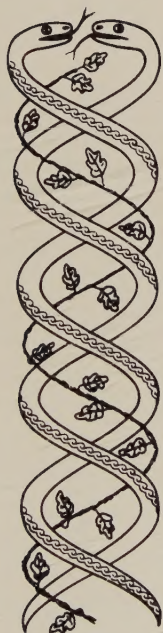


BOWEL REST AND COLONIC HEALING

Experimental studies in the rat

Peter Blomquist



Malmö 1984

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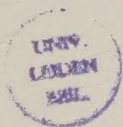
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To

Birgitta, Anna & Jonas

The present dissertation is based on the following studies:

- I. THE EFFECT OF RELATIVE BOWEL REST ON COLLAGEN IN THE COLONIC WALL. Studies in the rat.
Peter Blomquist, Hasse Jiborn & Bengt Zederfeldt.
Accepted for publication in Res Exp Med 1984.
- II. THE EFFECT OF RELATIVE BOWEL REST ON COLLAGEN METABOLISM AND SUTURE HOLDING CAPACITY IN THE COLONIC WALL. Studies in the rat.
Peter Blomquist, Hasse Jiborn & Bengt Zederfeldt.
Accepted for publication in Res Exp Med 1984.
- III. THE EFFECT OF RELATIVE BOWEL REST ON HEALING OF COLONIC ANASTOMOSES. Collagen synthesis and content in the colonic wall after left colon resection and anastomosis in the rat.
Peter Blomquist, Juhani Ahonen, Hasse Jiborn & Bengt Zederfeldt.
Accepted for publication in Acta Chir Scand 1984.
- IV. THE EFFECT OF RELATIVE BOWEL REST ON HEALING OF COLONIC ANASTOMOSES. Breaking strength and collagen in the colonic wall following left colon resection and anastomosis in the rat.
Peter Blomquist, Hasse Jiborn & Bengt Zederfeldt.
Accepted for publication in Acta Chir Scand 1984.
- V. THE EFFECT OF DIVERTING COLOSTOMY ON COLLAGEN METABOLISM IN THE COLONIC WALL. Studies in the rat.
Peter Blomquist, Hasse Jiborn & Bengt Zederfeldt.
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- VI. THE EFFECT OF DIVERTING COLOSTOMY ON BREAKING STRENGTH OF ANASTOMOSES AFTER LEFT COLON RESECTION. Studies in the rat.
Peter Blomquist, Hasse Jiborn & Bengt Zederfeldt.
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CONTENTS

Introduction	1
Purpose of the Present Investigation	3
Material and Methods	4
Comments on Material and Methods	8
Results	12
A. Non-Resected Animals	12
1. Low Residue Diet	12
2. Proximal Diverting Colostomy	13
B. Resected Animals	14
1. Low Residue Diet	14
2. Proximal Diverting Colostomy	17
General Discussion	18
Conclusions	25
Acknowledgements	27
References	28

Studies I-VI

INTRODUCTION

Resection and anastomosis of the colon, especially the lower left colon, is associated with a high incidence of anastomotic failure (Rousselot & Slattery, 1964; Goligher et al., 1970; Morgenstern et al., 1972), which is accompanied by a high mortality (Debas & Thomson, 1972; Schrock et al., 1973; Fielding et al., 1980).

Numerous factors, both systemic and local, are known to influence the healing of intestinal anastomoses (Muir, 1968; Hawley et al., 1970; Irvin & Goligher, 1973; Schrock et al., 1973; Jiborn, 1978; Ravitch et al., 1981; Khoury & Waxman, 1983). Such factors are: age and nutritional status of the patient, blood supply to the site of the anastomosis, tension over the anastomosis, suture technique, foreign materials around the anastomosis and peritoneal infection.

In retrospective clinical studies it is generally claimed that inadequate bowel preparation with faecal loading has an adverse effect on healing of colonic anastomoses (Goligher et al., 1970; Rosenberg et al., 1971). Irvin & Goligher (1973) showed that when the colon was poorly prepared the incidence of anastomotic dehiscence was 24 per cent compared to 7 per cent when the colon was well prepared. In experimental studies Jiborn et al. (1978a and 1980b) and Smith et al. (1983) found that faecal loading was accompanied by a high frequency of anastomotic complications following surgery on the left colon. Thus, there is strong evidence from both clinical and experimental studies that faecal loading is a factor of major importance for the breakdown of colonic anastomoses.

The mechanism whereby faecal loading affects colonic healing is not fully known. One possibility is that the faeces distends the anastomosis and thereby causes leakage and infection in and about the suture line. Whether the infection is the cause or the effect of leakage is, however, not known.

In experimental studies infection has an adverse effect on the healing of colonic anastomoses (Rothenberg et al., 1959; Yamakawa et al., 1971). Hawley et al. (1970) showed that anastomotic leakage and abscess formation is accompanied by an increased collagenolytic activity which might contribute to anastomotic breakdown.

Faecal loading can be diminished by different means. These include conventional bowel preparation with different kinds of enemas (Muir, 1968), whole bowel irrigation (Hewitt et al., 1973), oral mannitol bowel preparation (Hares & Alexander-Williams, 1982) and enteral nutrition with chemically defined low residue diets (Gurry & Ellis-Pegler, 1976).

Chemically defined diets that are low of residues were originally introduced in clinical practice to obtain bowel rest while giving nutritional support for the treatment of gastrointestinal fistulas and inflammatory bowel disease (Bury et al., 1971; Rocchio et al., 1974a; Rocchio et al., 1974b; Goode et al., 1976; O'Morain et al., 1980). Low residue diets are also used to provide colonic preparation for colonoscopy (Teague et al., 1973) and bowel surgery (Glotzer et al., 1973; Gurry & Ellis-Pegler, 1976; Hares & Alexander-Williams, 1982).

Diversion of the faecal content by a proximal colostomy is often used in emergency operations for obstructing carcinoma and diverticular disease or to prevent complications in colorectal anastomoses. However, a considerable incidence of anastomotic leakage despite proximal diversion has been reported by several authors (Goligher et al., 1970; Debas & Thomson, 1972; Schrock et al., 1973; Høier et al., 1975; Wara et al., 1981).

Thus, anastomotic complications in patients can be attributed to various detrimental factors, but mechanisms cannot easily be determined in clinical studies. In order to study single factors influencing healing in the gastro-intestinal

tract it is usually necessary to resort to experimental studies. We have developed an experimental model in the rat in our laboratory for such studies (Jiborn, 1978).

In the literature there are several studies concerning the influence of diminished bowel content on the intestinal mucosa (Fenyö & Hallberg, 1976; Janne et al., 1977; Rijke et al., 1979; Terpstra et al., 1981; Delvaux et al., 1983) but no studies on mechanical properties and collagen turnover in the colonic wall. Such studies have an interest since the mechanical strength of the intact bowel wall and its suture holding capacity is mainly dependent on the collagen in the submucosal layer (Halsted, 1887). Further, the development of anastomotic strength depends on the formation of fibrous tissue and collagen in the anastomosis (Cronin et al., 1968a; Irvin & Hunt, 1974).

PURPOSE OF THE PRESENT INVESTIGATION

was to study:

- the effect of relative bowel rest, obtained by feeding animals low residue diet, and
- the effect of total bowel rest, obtained by a proximal diverting colostomy, on
 - a) collagen metabolism in the unoperated colon,
 - b) suture holding capacity of the unoperated colonic wall,
 - c) collagen response in the colonic wall after left colon resection and anastomosis, and
 - d) suture holding capacity and breaking strength of left colon anastomoses.

MATERIAL AND METHODS

Experimental animals

Altogether 374 Wistar male rats (Møllegaards Breeding Centre Ltd, Denmark) weighing about 200 grams were used. Their weight development was recorded during the study periods.

Diets

The animals were fed either standard laboratory pellets or a chemically defined diet that is low of residues. The standard laboratory pellets contain per 100 g: 4 g fibres, 24 g protein, 5.5 g fat, 50 g carbohydrate, minerals and vitamins (1300 kJ). In studies (I-IV) on the effect of low residue diet the animals were fed a fibre free diet (Biosorbin^R MCT, Pfrimmer Meda; 100 g/100 g water) for four days prior to operation and during the study period. This diet contains per 100 g dry weight: 19 g protein, 19 g fat (MCT 15.2 g), 60 g carbohydrate, minerals and vitamins (2100 kJ). All animals had free access to their respective diet and water.

Caloric intake

In the first study (I) comparing low residue diet and standard laboratory diet the caloric intake was calculated by supplying a known amount of food and once a day weighing what was left. The remaining low residue diet was dried in a freeze-drier (Hetosicc type CD12) before weighing.

Operative procedures

Under general anaesthesia (chloral hydrate: 25 mg/100 g body weight i.p.) the abdomen was opened through a midline incision.

A left colon resection was performed 2.5 cm above the peritoneal reflection (Fig. 1). The anastomoses were made using a single layer of either inverting, continuous suture (study III and IV) or interrupted sutures (study VI). As suture material 7-0 silk (study III and IV) or 7-0 polypropylene (study VI) were used.

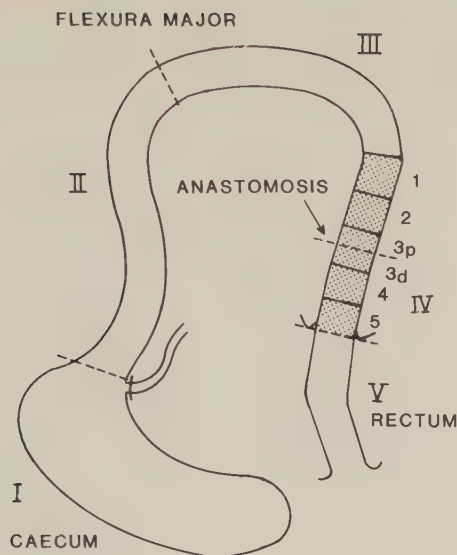


Fig. 1. Sites of anastomosis and studied colonic parts (I-V) and segments (1-5).

I: Caecum.

II: The proximal colon to the major flexure.

III: The distal colon to the anastomosis 2.5 cm above the peritoneal reflection.

IV: The distal colon below the anastomosis to the peritoneal reflection.

V: Rectum.

(Nomenclature according to Lindström et al., 1979).

In studies on colostomy (study V and VI) the colon was transected at the major flexure (Fig. 2). Great care was taken not to sever the marginal artery. The proximal end was brought out as a terminal colostomy in the upper part of the midline incision. The bowel wall was fixed to the fascia and skin with 7-0 polypropylene sutures. The distal end was closed with the same suture material after emptying the distal colon from faecal pellets.

The shamoperated animals (study V and VI) were submitted to laparotomy and mobilisation of colon.

Fascia and skin were closed separately with interrupted sutures of 4-0 polyester suture.

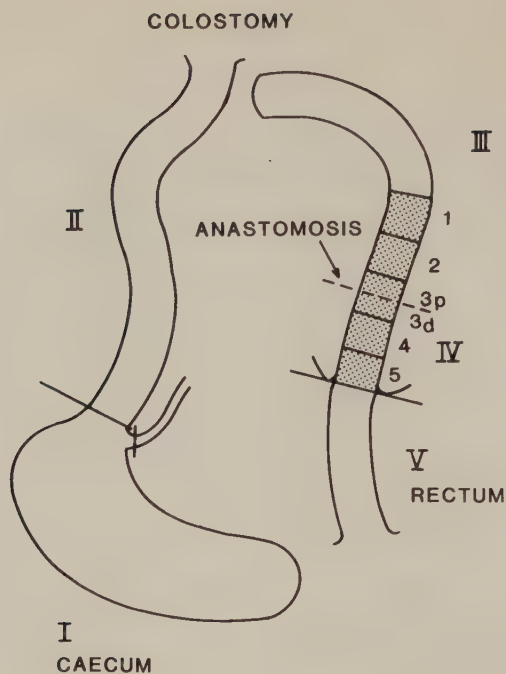


Fig. 2. Sites of colostomy, anastomosis and studied colonic parts (I-V) and segments (1-5). Symbols as in Figure 1.

Blood tests

Blood drawn from the aorta prior to sacrifice was analyzed for haemoglobin (g/l) and albumin (g/l). Haemoglobin concentration was determined as cyanmethaemoglobin as recommended by the International Committee for Standardization in Haematology (1965). Albumin concentration was determined using the method described by Lizana & Helsing (1974).

Gross appearance of the anastomosis

Gross complications, e.g. anastomotic dehiscence, perianastomotic abscesses and adhesions to the anastomotic region, were registered.

Gastrointestinal content

The weight of the content in different parts of the gastrointestinal tract was determined in studies on low residue diet (study I and III).

Colonic diameter

The diameter of the colon proximal and distal to the anastomosis was determined with a caliper (study IV).

Mechanical strength determination

Mechanical strength of different colonic segments was determined using breaking strength tests with a specially constructed tensiometer which provided a constantly increasing force from 0.03 to 0.05 N/s (in the interval from 0 to 3 N). The breaking strength of anastomoses was tested immediately after they were made (day 0) or during the first postoperative days with sutures left in place, to measure the suture holding capacity. On the seventh day the anastomotic strength was tested both with sutures left in place (study VI) and after removal of the sutures (study IV and VI).

Analysis of collagen metabolism and protein synthesis

After sacrifice of the animals by an overdose of ether the colon and rectum were removed and divided in 5 defined parts. From parts III and IV standardized, 10 mm segments were taken on both sides of the anastomosis (anastomotic segments were each 5 mm in length; Fig. 1 and 2).

The dry weight of each colonic part and segment was determined. The samples were hydrolyzed in 6 N HCl for 6 hours at 130°C. Collagen was determined as hydroxyproline (hypro), according to the method described by Stegemann & Stalder (1967) and Pikkarainen (1968). The collagen content (μg hypro/segment) and the collagen concentration (μg hypro/mg tissue dry weight) were calculated.

For determination of collagen and protein synthesis the animals were given 100 μCi (study III) or 200 μCi (study II and V) ^3H -proline (2-3-4-5- ^3H -L-proline; New England Nuclear, specific activity 100-120 Ci/mmol) intravenously in the tail 24 hours prior to sacrifice.

The incorporation of ^3H -proline into proteins and collagen was determined according to Juva and Prockop (1966) as applied in colonic healing by Jiborn et al. (1980a). The radioactivity was measured in a liquid scintillation counter (LKB Wallac 81 000). The protein synthesis (total dpm ^3H /g dry weight) and the collagen synthesis as specific activity of collagen (dpm ^3H -hypro/ μmol hypro) were calculated.

Statistical methods

In the statistical analysis of the results the mean (M), the standard deviation (SD) and the standard error of the means (SEM) were calculated. Comparison of the means were carried out by Student's t-test for unpaired observations.

COMMENTS ON MATERIAL AND METHODS

Experimental animals

Rats were used as experimental animals since they are suitable for studies of gastrointestinal healing and previously used for such studies in our laboratory.

Suture technique and suture material

The anastomoses were made with one layer of inverting sutures, either continuous or interrupted. When comparing the effect of low residue diet and standard laboratory diet a continuous suture was used (study III and IV), since it was shown by Jiborn et al. (1980b) that this technique was associated with a high frequency of anastomotic complications and an enhanced rate of collagen turnover.

When investigating the effect of faecal diversion on anastomotic healing (study VI) interrupted sutures were used, since we found that the number and the position of the stitches were easier to standardize with this technique than when continuous suture was used.

Silk was used as suture material in anastomoses performed with a continuous suture technique (study III and IV), since this material was easy to handle. In study VI polypropylene, a monofilament synthetic material, was used, since this suture material could be removed from the tissues with less trauma than silk sutures.

Mechanical strength determination

Mechanical strength of the intact bowel wall and of colonic anastomosis can be determined either as bursting pressure or as breaking strength (Fellows et al., 1951; Herrmann et al., 1964; Cronin et al., 1968a; Irvin & Hunt, 1974; Wise et al., 1975; Jiborn et al., 1978b; Jiborn et al., 1978c).

In bursting strength tests the values are extremely low the first four postoperative days. From the seventh day on the rupture usually occurs outside the anastomosis (Nelsen & Anders, 1966; Herrmann et al., 1964; Yale & Gemert, 1971; Jiborn et al., 1978b). Jiborn et al. (1978c) concluded that bursting strength determination only gives limited information about the process of healing in the bowel and therefore such tests have not been used in the present investigation.

In breaking strength tests with the sutures removed from the anastomosis the values are low on the fourth day after operation, but from that time until the tenth day the strength increases rapidly (Irvin & Edwards, 1973; Hastings et al., 1975; Jiborn et al., 1978c). Further, in breaking strength tests the rupture occurs in the anastomotic line during the first postoperative weeks in contrast to bursting strength tests.

During the first days after making an anastomosis the mechanical strength depends almost entirely on suture strength and on the ability of the bowel wall to withstand the tearing forces of the sutures, e.g. the suture holding capacity (Howes et al., 1929). As the anastomosis heals by fibrous tissue formation the role of the sutures becomes successive-

ly smaller. Determination of mechanical strength with sutures in place gives a measure of suture holding capacity and is of interest during the early phase of healing, when anastomotic dehiscence is most common.

When comparing the effect of low residue diet and standard laboratory diet on mechanical strength development the first postoperative week a continuous, inverting suture technique was used (study IV). On day 0 to 4 the suture was left in place. On the seventh postoperative day the suture in animals fed standard laboratory diet had disappeared or hang loose in the colonic lumen while it stayed intact in animals on low residue diet. To have comparable conditions for testing strength, the suture material was removed in both groups of animals on the seventh day.

Sampling technique

Great care was taken to standardize the size and locations of the colonic samples. The collagen was determined in anatomically defined parts to see if this corresponds with the results from smaller, but well standardized segments from the colonic wall. Since the results correlate well the data from the smaller colonic segments are considered representative.

Collagen and protein determinations

Collagen was studied by determination of hydroxyproline which is almost uniquely found in collagen and forms about 14 weight per cent of the collagen molecule (Eastoe, 1967). In the present investigation both the collagen content and the collagen concentration were calculated. Some authors (Woessner, 1968; Irvin & Hunt, 1974; Stromberg & Klein, 1982) advocate determination of collagen content. However, collagen concentration, expressed in relation to tissue dry weight, is of interest together with collagen content, since evaluation of changes in other components than collagen can be made.

Detailed analysis of collagen metabolism requires determination of collagen synthesis or lysis in addition to the net amount of collagen. Since hydroxyproline as such is not incorporated into collagen (Stetten, 1949) it is possible to study the metabolism of collagen by using radioactively labelled proline as precursor. Synthesis of collagen can be studied by ^3H -proline using a pulse-labelling technique and determination of ^3H -hydroxyproline (Cronin et al., 1968b). Evaluation of lysis requires prelabelling of the collagen by the same precursor from the early life of the animal (Klein, 1968). Both methods have been extensively used for studying collagen metabolism in intestinal healing. The prelabelling technique requires much higher dosage of radioactive proline and is consequently more expensive. Further, since there is evidence for reutilization of radioactive proline after operative trauma (Irvin & Hunt, 1974) results obtained can be difficult to evaluate.

The pulse-labelling technique was preferred in this investigation. The animals were given either 100 μCi (study III) or 200 μCi ^3H -proline (study II and V). The higher dosage of ^3H -proline was used to ensure a high yield of hydroxyproline radioactivity in the unoperated animals with a low metabolic rate of collagen. The incorporation time of 24 hours used in the present study was found by Jiborn et al. (1980a) to be the optimal time for in vivo incorporation.

The specific activity of ^3H -hydroxyproline gives a measure of the newly synthesized collagen. The total radioactivity reflects the synthesis of all proteins including collagen.

RESULTS

A. Non-resected animals

1. Low residue diet (study I and II)

Nutritional status

Nutritional parameters as caloric intake, weight development, albumin and haemoglobin concentration in the plasma were similar in animals fed low residue diet and standard laboratory diet during the study period which lasted for 14 days.

Bowel content

Low residue diet reduced the colonic and caecal contents by 90 per cent ($p < 0.001$) and 45 per cent ($p < 0.01$), respectively, compared to standard laboratory diet. The content was unchanged in the small bowel but increased in the stomach ($p < 0.05$).

Tissue dry weight, collagen content and collagen concentration in the colon on days 4, 11 and 18

In the colon (parts II, III and IV) the collagen content in the colonic wall was unchanged in animals fed low residue diet. The tissue dry weight was decreased by on average one third. Consequently, the collagen concentration was markedly increased.

In the rectum (part V) there were no marked changes in collagen content and concentration or tissue dry weight in animals fed low residue diet.

In the caecum (part I) there was a significant increase of collagen content and of tissue dry weight in animals fed low residue diet. The collagen concentration was decreased.

The collagen and protein synthesis in the colon on day 11

The collagen synthesis was significantly reduced in the colon (parts II, III and IV) but unchanged in caecum (part I) and rectum (part V) in animals fed low residue diet.

The protein synthesis was increased in the caecum (part I) and the proximal part of colon (part II) but decreased in the distal part of colon (parts III and IV) and rectum (part V).

Mechanical strength of the colon on days 4, 11 and 18

The suture holding capacity, e.g. the strength immediately after transection and a standardized end-to-end anastomosis, in the middle of each colonic part II, III and IV, was equal in animals on low residue diet and standard laboratory diet.

2. Proximal diverting colostomy (study V and VI)

Nutritional status

The postoperative weight development was delayed in the animals with a proximal colostomy compared to the shamoperated animals (on the 28th day: 293 ± 11 g and 317 ± 18 g, respectively; $p < 0.01$).

The albumin and haemoglobin concentration were equal in the two groups of animals.

Tissue dry weight, collagen content and collagen concentration in the colon on days 4, 11 and 28

The dry weight was significantly decreased distal to the colostomy site in parts III to V already after a period of 11 days in comparison with shamoperated controls. At that time the collagen content was decreased only in rectum (part V) but after 28 days the collagen content was markedly decreased in all parts of the excluded colon. The collagen concentration, however, was significantly increased distal

to the colostomy site due to the relatively more marked reduction in tissue dry weight.

In the colonic part proximal to the colostomy (part II) there was also a decrease in tissue dry weight and collagen content but less marked than in the distal parts.

The collagen and protein synthesis in the colon on day 11
The collagen and protein synthesis were significantly decreased distal but unchanged proximal to the colostomy site compared with the shamoperated controls.

Mechanical strength of the colon distal to the colostomy on day 7

The suture holding capacity, e.g. the strength immediately after performing a left colon anastomosis 7 days after a proximal colostomy, was approximately 35 per cent higher ($p < 0.05$) than in shamoperated animals.

B. Resected animals

1. Low residue diet (study III and IV)

Nutritional status

All animals had an immediate postoperative weight loss of 5 per cent. Animals fed low residue diet started to gain weight earlier than animals fed standard laboratory diet, but after 14 days the weight was similar in the two groups.

There were no significant differences in albumin or haemoglobin concentration between the two groups of animals.

Gross appearance of the anastomosis

There were few complications that could be attributed to defective anastomotic healing. No animals out of 198 died. In study IV 9 out of 112 animals (8%) had small perianastomotic abscesses. Eight of these were found on the third and

fourth postoperative day (three in animals on low residue diet and five in animals on standard laboratory diet). All nine abscesses were considered as anastomotic complications and these animals were excluded from further study. In animals fed standard laboratory diet adhesions to the anastomotic region were common but in the low residue group there were practically no adhesions.

Colonic diameter

The bowel was wider on both sides of the anastomosis in animals fed standard laboratory diet than in animals on low residue diet and in both groups wider on the proximal than on the distal side.

Bowel content

The weight of the colonic content was on the seventh postoperative day significantly lower ($p < 0.001$) in animals fed low residue diet than in animals on standard laboratory diet (0.7 ± 0.4 and 2.1 ± 0.6 g, respectively).

Tissue dry weight, collagen content and collagen concentration in the colonic wall on days 2-14 after left colon resection and anastomosis

From the second to the seventh postoperative day there was a gradual increase of collagen content in animals on standard laboratory diet compared with unoperated animals. On the seventh day both the collagen content and the tissue dry weight were significantly increased and the collagen concentration was decreased in the colonic parts III and IV. The observed changes were most pronounced in the close vicinity of the anastomosis, especially on its proximal side. Collagen content remained on a significantly increased level on day 14 but the difference against controls was less.

In animals on low residue diet the collagen content and the dry weight were on a lower level than in animals on standard laboratory diet. In fact the dry weight was decreased in parts II, III and V compared with unoperated controls on day

7. On the fourteenth day the collagen content in the low residue diet group was similar to the controls. The collagen concentration, however, was decreased in the anastomotic region on day 7 in both groups of resected animals compared with the unoperated controls due to the relatively marked increase in tissue dry weight.

The collagen synthesis in the colonic wall on day 7 after left colon resection and anastomosis

On the seventh postoperative day the collagen synthesis was significantly increased in the three segments proximal and in the two segments distal to the anastomosis in animals on standard laboratory diet compared with unoperated controls.

In animals on low residue diet the collagen synthesis was significantly lower in all segments than in animals fed standard laboratory diet. In animals on low residue diet the collagen synthesis was increased only in the two anastomotic segments compared with the controls.

Mechanical strength and collagen content of the colon on days 0-7 after left colon resection and anastomosis

In both groups of animals there was a similar and significant decrease of suture holding capacity on the first two postoperative days. From that time there was a significant gain in strength to the seventh postoperative day when anastomotic strength with the sutures removed had reached the suture holding capacity of day 0, e.g. the strength of a newly made anastomosis.

In all tests the break occurred in the anastomotic line. The suture came loose from the proximal side of the anastomosis in 27 out of 48 (56%) animals on day 0 and 1 and in 35 out of 40 (88%) on day 2 to 4.

In animals on standard laboratory diet there was in this study an increase of collagen content in the anastomotic segments from the third day on amounting to approximately

2.5 times on day 7. In animals fed low residue diet there was a significant decrease of collagen content on the second day but from the fourth day on an increase was found amounting to about 1.4 times on day 7, which was significantly lower than that observed in animals on standard laboratory diet.

2. Proximal diverting colostomy (study VI)

Nutritional status

The weight increase was equally delayed on the seventh post-operative day in animals with a left colonic anastomosis, with or without a proximal diverting colostomy, compared to shamoperated animals.

No blood samples were taken for analysis.

Gross appearance of the anastomosis

In animals without a diverting colostomy three out of sixteen animals showed signs of anastomotic leakage with small perianastomotic abscesses against none in animals with a colostomy. Abscesses were considered as anastomotic complications and therefore the three animals were excluded from further study. Adhesions were common in animals without a colostomy but were not seen in those with a colostomy.

Collagen content and mechanical strength of the colon on day 7 after left colon resection and anastomosis

In animals without a colostomy the collagen content was significantly higher in the colonic part III and in segments 1-4 than in animals with a colostomy, where the collagen content was unchanged in all colonic parts and segments compared to shamoperated animals.

The anastomotic strength was approximately 45 per cent lower ($p < 0.001$) in animals with a colostomy than in those without a colostomy. In each group the strength without sutures was not statistically different from that with sutures left in place.

GENERAL DISCUSSION

The strength of a gastro-intestinal anastomosis is during the first postoperative days completely dependent on suture strength and the ability of the bowel wall to support the sutures, e.g. the suture holding capacity (Howes et al., 1929; Cronin et al., 1968a). The strength of the bowel wall is determined by the fibrous protein collagen (Peacock & van Winkle, 1976), mainly located in the submucosal layer (Halsted, 1887). Anastomotic healing means that new fibrous tissue bridges the wall defect and with time this tissue and its collagen content becomes the dominating factor determining anastomotic strength (Peacock, 1967; Irvin & Hunt, 1974).

Anastomotic complications could be due to cutting through of sutures and/or to impaired connective tissue formation over the anastomosis. Both factors are related to collagen metabolism. Collagen metabolism in and around an anastomosis is thus of central importance for the fate of an anastomosis both in the early and late phases of healing.

From experimental studies (Jiborn et al., 1978a; Jiborn et al., 1980b; Smith et al., 1983) and clinical observations (Goligher et al., 1970; Rosenberg et al., 1971; Irvin & Goligher, 1973) it seems evident that faecal loading causes an increased complication rate after colonic surgery. Analysis of collagen metabolism and anastomotic strength in relation to bowel content should allow evaluation of mechanisms behind these complications.

To avoid faecal loading two different methods were used: enteral nutrition with a low residue diet (Biosorbin^R MCT) and proximal faecal diversion with a colostomy.

The low residue diet induced a state of relative bowel rest, e.g. the colonic content was markedly but not completely reduced with passage of stools through the colon although to

a lesser extent. There was no faecal stagnation seen above the anastomoses, which was common when animals were fed standard laboratory diet. Total bowel rest was achieved by constructing a terminal colostomy and by emptying the excluded colon of faecal contents at the operation. Thus, by this method no passage of stools occurred in the colon distal to the colostomy.

The nutritional variables studied were principally unchanged after institution of bowel rest and consequently the observed differences in collagen metabolism and mechanical properties were not due to impairment of the general nutritional state of the animals.

Within a few days of relative or total bowel rest there was a marked decrease in non-collagenous substances, e.g. mucosal cells and muscle tissue, which constitute approximately 90 per cent of the tissue dry weight. These findings are consistent with previous reports that low residue diet and proximal faecal diversion induce loss of cells in the mucosal layer of the bowel (Janne et al., 1977; Rijke et al., 1979; Terpstra et al., 1981; Delvaux et al., 1983).

Several factors are proposed to influence cell replacement in the colon, e.g. gastrin (Ryan et al., 1979), bacterial flora (Winitz et al., 1970) and physical stimulation of food residues in the bowel lumen (Stragand & Hagemann, 1977). In some studies low residue diet reduced the colonic bacterial flora (Winitz et al., 1970; Bounous & Devroede, 1974) but in other studies no change of the faecal flora was found (Axelsson & Justesen, 1977; Arabi et al., 1978). The findings in the present investigation indicate that intraluminal stimulation of food residues is a factor of importance in regulating tissue composition of the colonic wall. However, eventual influence by the faecal flora cannot be evaluated from this study.

Contrary to the decrease in non-collagenous substances the collagen content in the colonic wall remained unchanged in relative bowel rest over a period of 18 days. Total bowel rest, however, led to a reduction not only of non-collagenous components but also of collagen content in the rectum after a period of 18 days and in all excluded parts of the colon after 28 days. Thus, the changes in non-collagenous substances occur earlier than the changes in collagen content after introducing bowel rest.

The collagen concentration in the colonic wall showed an opposite pattern of change compared with collagen content, after introducing bowel rest, with a marked increase. This indicates a relatively greater change in non-collagenous substances than in collagen content. These findings confirm the opinion of Irvin & Hunt (1974) that collagen concentration can be misleading as a measure of the actual collagen amount. However, collagen concentration allows evaluation of quantitative changes in other components of the tissue than collagen.

The unchanged and moderately reduced collagen content in relative and total bowel rest, respectively, does not mean an unchanged collagen metabolism. To the contrary, the collagen synthesis was markedly reduced when studied after a period of 11 days of bowel rest. The unchanged collagen content despite reduced synthesis of collagen must mean that lysis of collagen was diminished both with relative and total bowel rest.

The factors that regulate the collagen turnover in the intestinal wall are not fully known, but the present findings indicate that the stimulation of intraluminal bulk is a factor of importance.

The suture holding capacity of the colonic wall was unchanged with relative bowel rest but significantly increased with total bowel rest after a period of 7 days. Thus, total bowel

rest increases the ability of the excluded bowel wall to withstand the tearing forces of the sutures. As the amount of collagen was unchanged in both types of bowel rest at that time, there was no regular correlation between suture holding capacity and collagen content. One explanation for the lack of relation might be that the quality of collagen is changed with total bowel rest but unchanged with relative bowel rest. It may be speculated that the lower turnover rate of collagen in total bowel rest is accompanied by higher maturity of the collagen compartment and thereby increased bowel strength. Further studies on this subject are planned.

The findings of marked depression of collagen turnover in the intact colonic wall after introducing bowel rest, raised the question if the healing of an anastomosis might be impaired, since collagen formation is a central factor for anastomotic strength development.

In previous studies Jiborn et al. (1980b) found marked increase of collagen synthesis in the colonic wall after left colon resection in animals on standard laboratory diet. The increase in collagen synthesis was most marked and extended proximally in colon when continuous suture was used, a technique that led to stagnation of bowel content above the anastomosis. The present investigation confirms these findings of marked and asymmetrical increase of collagen synthesis in relation to the anastomotic line in animals fed standard laboratory diet.

With relative bowel rest the postoperative increase of collagen synthesis and collagen content was less marked. In fact, the increase of collagen synthesis and content was confined to the immediate anastomotic region, and the more distant collagen response in the colon was eliminated. Furthermore, the increase of collagen content was of short duration, returning to normal on the fourteenth postoperative day with relative bowel rest. The reduction of collagen

concentration in the anastomotic area was a consequence of smaller increase in collagen than in non-collagenous substances.

Total bowel rest completely eliminated both the local and the distant postoperative increase of collagen content in the colonic wall. Although collagen synthesis was not studied it can be deduced that collagen turnover must have been reduced.

During the first postoperative week mechanical strength was comparable in animals on standard laboratory diet and low residue diet. In both groups strength decreased during the first postoperative days, reaching its lowest point on day 2.

Using the data from Jiborn et al. (1978c) for strength of the intact left colon (6.1 N) it can be calculated that the strength of a newly made anastomosis with our technique is only 30 per cent of that of the intact bowel wall. At its lowest point on day 2 the strength is approximately 20 per cent of the intact bowel wall, which is in accordance with the findings reported by Herrmann et al. (1964). The lowest values of anastomotic strength were on the same level as those found for small intestinal anastomoses by Jönsson et al. (1983).

The initial decrease in strength means that suture holding capacity decreased. This indicates changes in collagen quality since collagen quantity remained the same.

After the second day the strength gradually increased, reaching the values of day 0 after 7 days. Some of this regain of strength may be due to increase of suture holding capacity but formation of new tissue with collagen deposition in the anastomosis is probably the major factor. In the tests on the seventh day all strength is due to new tissue in the anastomosis since the sutures were then removed.

Total bowel rest, obtained by a proximal colostomy, led to a marked reduction of anastomotic strength on the seventh postoperative day. The finding of similar strength when tests were performed with and without sutures shows that suture support is of little or no importance to the strength of a colonic anastomosis one week after surgery.

The changes in anastomotic strength in relative and total bowel rest did not correlate regularly with collagen content. One explanation may be that collagen content is measured in anastomotic segments and thus represents both the anastomotic line and the adjacent bowel wall. Measurement of changes solely in the anastomotic line could not be performed. It is, however, also possible that the quality of the collagen around the sutures and in the anastomotic line has undergone changes. This has so far not been studied.

Jiborn et al. (1980b) found that the synthesis of collagen was more increased in animals with colonic dilatation and marked faecal stagnation than in animals with uncomplicated healing. Faecal loading proximal to an experimental colonic stenosis is also accompanied by an enhanced turnover rate of collagen and an increased collagen deposition (Blomquist et al., 1979). Diminished turnover rate of collagen in relative and total bowel rest could represent a less complicated healing pattern rather than being a sign of impaired healing.

Gross anastomotic complications as dehiscence and abscess formation were rare in all experiments. However, anastomotic adhesions were common after surgery in animals fed standard laboratory diet but were seldom seen in animals on low residue diet or in animals with a diverting colostomy. On the assumption that adhesion formation is a result of bacterial contamination and/or increased inflammatory response in and about the anastomotic line, these findings suggest that bowel rest gives a more uncomplicated healing. The observed reduction of anastomotic strength in animals with total bowel rest should then rather represent less complicated

than impaired healing. It remains an open question if strength determinations in this situation is a relevant measure of anastomotic quality.

Our findings imply that the reduced collagen turnover rate, as a consequence of relative bowel rest, does not impair suture holding capacity or anastomotic strength development in the early phase of healing. Diminished collagen response and reduced anastomotic strength in animals with total bowel rest might mean an impaired healing but could as well be interpreted as a sign of less complicated healing. Further studies are necessary to elucidate this problem.

CONCLUSIONS

The present investigation has shown that:

I. in the non-resected colon:

relative bowel rest:

- reduces non-collagenous substances,
e.g. mucosa and muscle tissue,
- has no effect on collagen content,
- reduces collagen and protein synthesis,
- has no effect on suture holding capacity, and that

total bowel rest:

- reduces non-collagenous substances,
- decreases collagen content in course of time,
- reduces collagen and protein synthesis,
- increases suture holding capacity.

II. after left colon resection and anastomosis:

relative bowel rest:

- reduces and confines the postoperative increase of collagen synthesis and collagen content to the immediate anastomotic region,
- has minor influence on suture holding capacity and anastomotic strength development, and that

total bowel rest:

- eliminates the postoperative increase of collagen content, and
- reduces anastomotic strength.

Thus it can be deduced from the present investigation that the intraluminal content is of significance for collagen turnover and anastomotic strength development in colon. Relative bowel rest, obtained by a low residue diet (Biosor-

bin^R MCT), does not impair suture holding capacity or anastomotic strength, despite reduced turnover rate of collagen. Total bowel rest, obtained by a proximal colostomy, results in decreased collagen response and reduced anastomotic strength which might mean impairment of healing but could as well represent less complicated healing.

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I

THE EFFECT OF RELATIVE BOWEL REST ON

COLLAGEN IN THE COLONIC WALL

Studies in the rat

Peter Blomquist, Hasse Jiborn & Bengt Zederfeldt

ABSTRACT

In previous studies it was shown that diminished colonic content pre- and postoperatively, obtained by feeding rats low residue diet, results in less marked postoperative disturbances in collagen metabolism than in animals fed standard laboratory diet.

In the present investigation the effect of relative bowel rest on collagen content and collagen concentration in the intact colonic wall was studied by feeding unoperated rats low residue diet. It was found that the colonic and caecal contents were reduced significantly and that this primarily affected the non-collagenous substances in the colon with a marked decrease, while the collagen content was unchanged. In the caecum both the collagen and the non-collagenous contents were significantly increased. Thus the pattern of change in biochemical response varied in different parts of the large intestine after institution of bowel rest. Further, the present data imply that collagen concentration can be misleading as a measure of the actual collagen amount.

INTRODUCTION

Enteral nutrition with chemically defined diets that are low of residues has been introduced to obtain bowel rest while maintaining nutritional support in the treatment of inflammatory bowel disease and preoperatively in intestinal cancer (15).

In previous experimental studies we have found (25) that diminished colonic content pre- and postoperatively, obtained by feeding rats low residue diet (Biosorbin^RMCT), results in less marked postoperative disturbances in collagen metabolism in the colon than when animals are fed standard laboratory diet.

The aim of the present investigation was to study the effect of relative bowel rest on collagen content and collagen concentration in the intact colonic wall by feeding growing, unoperated rats low residue diet.

MATERIAL AND METHODS

Sixty-six Wistar male rats weighing about 200 g were used. They were randomly allocated to one of two groups that were kept in separate cages. In all experiments one group (LR-group) was fed a fibre free, chemically defined diet (Biosorbin^RMCT, 100 g/ 100 g water) containing per 100 g dry weight: 19 g protein, 19 g fat (MCT 15.2 g), 60 g carbohydrate, minerals and vitamins (2100 kJ). The other group (SL-group) served as controls and was given standard laboratory diet containing 4 g fibres per 100 g: 24 g protein, 5.5 g fat, 50 g carbohydrate, minerals and vitamins (1300 kJ). Both groups of animals had free access to their respective diet and water.

Experiment I (gastrointestinal content)

Six rats were used in each group and after 4 days on the respective diet they were sacrificed. The weight of the content in the different parts of the gastrointestinal tract was determined.

Experiment II (caloric intake and weight development)

Five rats were used in each group and before the study period, which lasted for 14 days, they were allowed to adapt to their respective diet for 3 days. Their weight was checked daily. The caloric intake was calculated by supplying a known amount of food and once a day weighing what was left. The remaining LR-diet was dried in a freeze-drier (Hetosicc type CD 12) before weighing.

Experiment III (collagen concentration and content)

The LR-group, consisting of 29 rats, was studied for 4, 11

and 18 days (7, 15 and 7 rats, respectively) and the weight development was recorded. The SL-group consisted of 15 rats.

The animals were sacrificed by an overdose of ether. Blood drawn from the aorta was analyzed for haemoglobin (g/l) and albumin (g/l). The colon and rectum were removed and divided in 5 defined parts (Fig. 1). The dry weight of each part was determined. Collagen was determined as hydroxyproline (hydro), according to the method described by Stegemann and

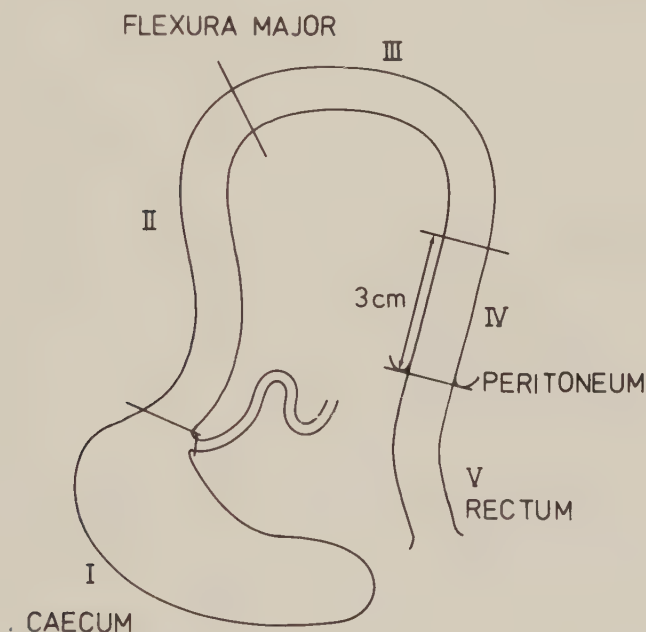


Fig. 1. Sites of colonic parts for analysis (I-V).

- I: Caecum.
- II: Pars proximalis coli to flexura major coli.
- III: Pars distalis coli to 3 cm above the peritoneal reflection.
- IV: Pars distalis coli below part III to the peritoneal reflection.
- V: Rectum.

(Nomenclature according to Lindström et al. (16)).

Stalder (22) and Pikkarainen (18). The hydroxyproline concentration (μg hypro/mg tissue dry weight) and the hydroxyproline content (μg hypro/part) were calculated.

Statistical methods

In the statistical analysis of the results the mean and the standard deviation were calculated. Comparisons of the means were carried out by Student's t-test for unpaired observations. Probability levels are represented by the following signs in table and figures: * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$.

RESULTS

Experiment I

The gastrointestinal content in various parts is given in Table I. The low residue diet reduces the colonic and caecal contents significantly (90% and 45% respectively) and increases the content in the stomach compared to standard laboratory diet.

TABLE I. Gastrointestinal contents in the low residue diet (LR) group and in the standard laboratory diet (SL) group.

Organ	Weight of the contents (g)	
	LR-group n=6	SL-group n=6
Stomach	4.9 ± 3.3	$0.9 \pm 0.2^*$
Small bowel	1.9 ± 0.9	2.5 ± 0.8
Caecum	2.4 ± 1.0	$4.4 \pm 0.6^{**}$
Colon exl caecum	0.2 ± 0.2	$1.8 \pm 0.8^{***}$

Asterisks denote level of statistical significance (see Statistical Methods).

Experiment II

The caloric intake is equal during the experimental period of 14 days for the SL- and LR-groups (1.17 ± 0.13 and 1.38 ± 0.21 kJ g body weight/day, respectively).

There is no difference in weight development between the SL- and LR-groups during the period of study (Fig. 2).

Experiment III

The weight development of animals fed LR-diet is similar to that in experiment II.

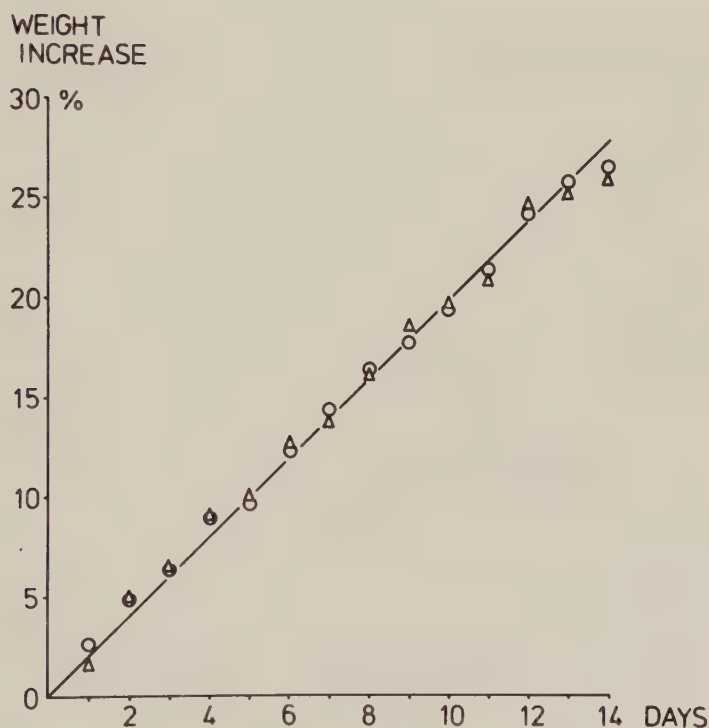


Fig. 2. Weight development in per cent of body weight for animals fed low residue diet (Δ) (day 0: 230^{+5} g) and standard laboratory diet (O) (day 0: 225^{+10} g).

Haemoglobin concentration is significantly increased after 4 days on LR-diet compared to SL-diet (15.3 ± 1.7 g/l and 10.7 ± 1.4 g/l respectively). On the other days studied there are no significant differences.

There are no significant differences in albumin concentration for the two groups.

The dry weight of the 5 different colonic parts is given in Fig. 3 as a ratio between the two groups. The dry weights of the colon (parts II, III and IV) are significantly decreased for the LR-group, unchanged for the rectum (part V) but significantly increased for the caecum (part I) in the LR-group.

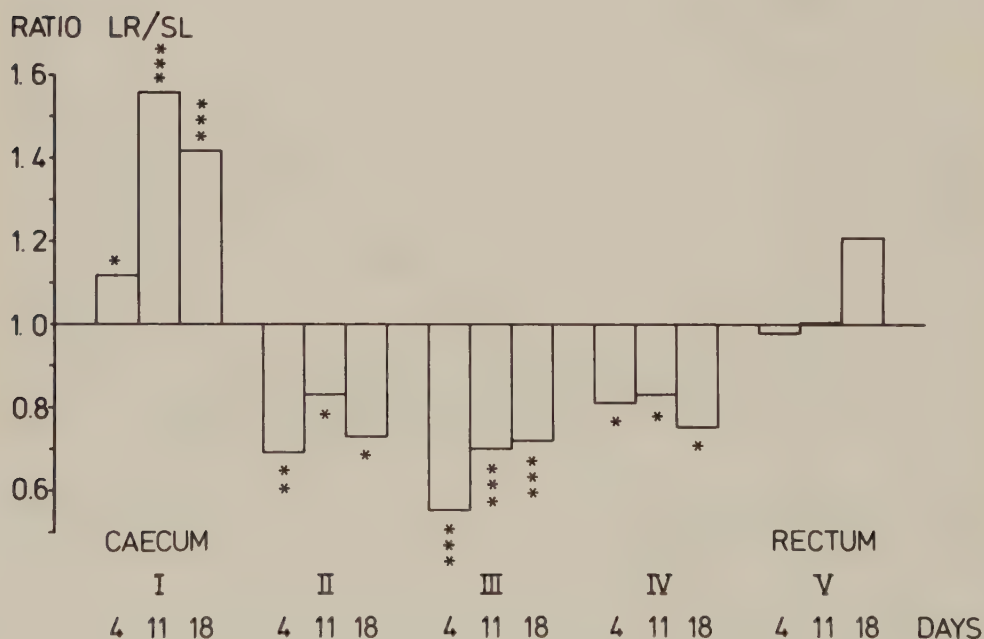


Fig. 3. Dry weight of the different colonic parts after 4, 11 and 18 days on low residue diet (LR) expressed as a ratio to standard laboratory diet (SL). Asterisks denote level of statistical significance (see Statistical methods).

RATIO LR/SL

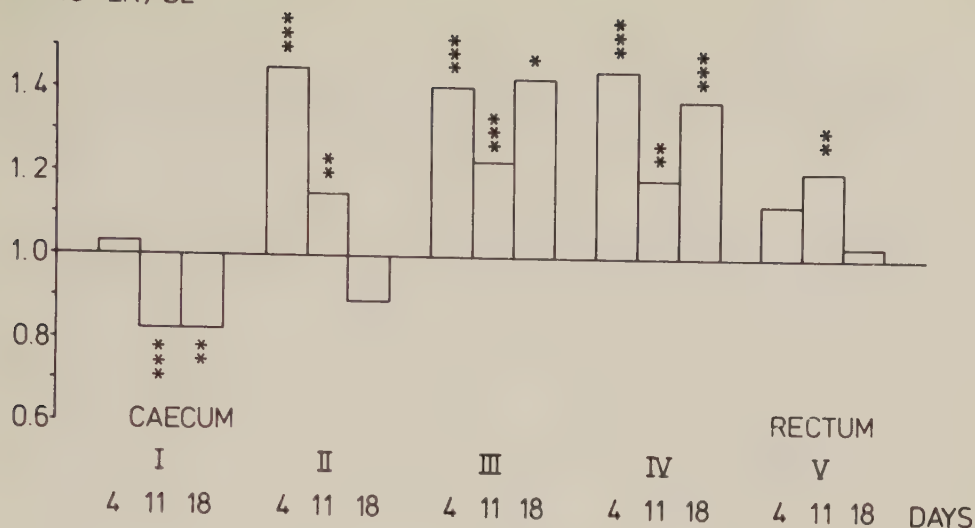


Fig. 4. Collagen concentration in the different colonic parts after 4, 11 and 18 days on low residue diet (LR) expressed as a ratio to standard laboratory diet (SL). Asterisks denote level of statistical significance (see Statistical Methods).

RATIO LR/SL

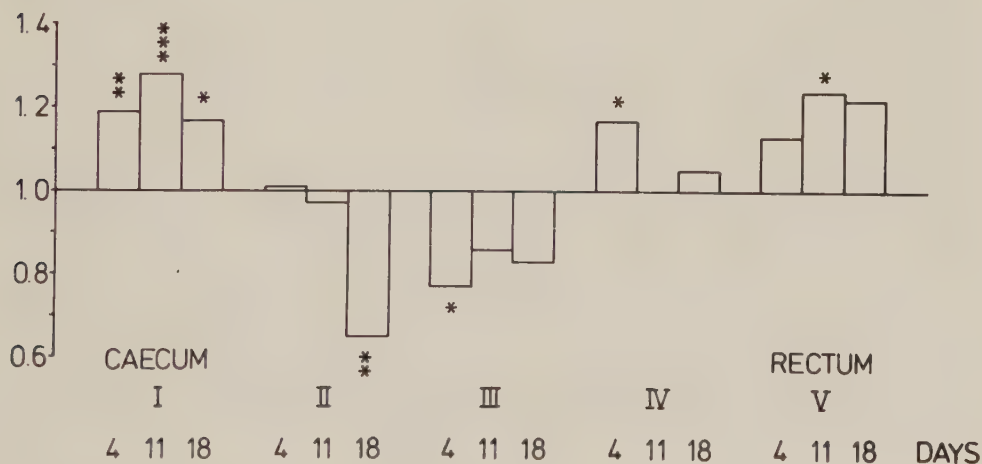


Fig. 5. Collagen content in the different colonic parts after 4, 11 and 18 days on low residue diet (LR) expressed as a ratio to standard laboratory diet (SL). Asterisks denote level of statistical significance (see Statistical Methods).

The hydroxyproline concentration in the 5 different colonic parts is given in Fig. 4 as a ratio between the two groups. In the colon (parts II, III and IV) there is a significant increase of hydroxyproline concentration in the LR-groups compared to the SL-group (except on day 18 in part II). There is an increase of hydroxyproline concentration in the rectum (part V) after 11 days on LR-diet compared to SL-diet while in the caecum (part I) there is a significant decrease after the eleventh day on LR-diet.

The hydroxyproline content in the 5 different colonic parts is given in Fig. 5 as a ratio between the two groups. There are no systematic differences in the rectum and colon of the LR-group compared to the SL-group. However, in the caecum there is a significant increase of hydroxyproline content for the LR-group.

DISCUSSION

In previous studies we have found that resection and anastomosis of the left colon in the rat leads to marked changes in collagen metabolism in the colonic wall proximal to the anastomosis (14). The disturbances in collagen turnover after left colon resection and anastomosis are less marked in animals fed low residue diet (LR-diet), which reduces the colonic and caecal content compared to standard laboratory diet (SL-diet) (25,26). Bowel rest by LR-diet may also influence collagen metabolism in the intact, unoperated colonic wall. Such an influence might be of interest because the mechanical strength of the intact bowel and its suture bearing capacity is almost completely dependent on the collagen in the connective tissue in the submucosa (7).

Low residue diets given orally have been discussed as a way to provide colonic preparation for colonoscopy (23) and bowel surgery (4,6,8) because of its low residue. Further, LR-diets have been proposed for the treatment of gastroin-

testinal fistulas and inflammatory bowel disease in several studies (1,5,17,20,21) since they provide good nutrition while permitting the bowel to rest.

In this study the colonic and caecal contents are reduced by 90% and 45% respectively when the animals are fed LR-diet. The caecum still functions as a reservoir and most of the remaining content is located there. The consistency of the content in the caecum is semi-fluid, adheres to the mucosa and contains a lot of fragments of hair. In the LR-group the caecum is to some extent reduced in volume and gives a shrunken impression.

Nutritional parameters as caloric intake, weight development, albumin and haemoglobin concentration in the plasma are similar to the control group. The increase of haemoglobin concentration on the fourth day on LR-diet is probably due to hyperosmolarity of the food in the gastrointestinal tract leading to withdrawal of fluid from the intravascular compartment, which later on is compensated.

Collagen was studied by determination of hydroxyproline which is almost exclusively found in collagen and forms about 14 weight per cent of the collagen molecule (3). In some previous studies on colonic healing collagen has been expressed in relation to tissue dry weight, e.g. collagen concentration (2,9,13). Irvin and Hunt (11) have advocated determination of content instead of concentration in collagen studies. One problem in studies of collagen content is, however, to ensure standardized biopsies for analysis. In this study well defined parts of the colon are used and both the content and the concentration of collagen are calculated.

In the colon, e.g. parts II, III and IV, where the reduction of faecal content is most pronounced on LR-diet, the collagen content in the colonic wall is unchanged but the collagen concentration is markedly increased. Thus, the collagen

concentration does not represent the actual collagen amount. The increase of collagen concentration is due to decrease of tissue dry weight by on average one third (Fig. 3).

Our findings imply that non-collagenous components, e.g. mucosal cells, ground substances and muscle tissue, are markedly reduced already after a few days on LR-diet. These components, according to our data constitute approximately 90% of the dry weight. After total bowel rest obtained by a transverse colostomy, the left colon undergoes progressive atrophy, with loss of cells principally in the mucosal layer of the bowel (19,24). The presence of nutrients in the gastrointestinal tract is a stimulus for cell renewal and mucosal growth, while diminished content by an elemental diet induces colonic mucosal atrophy (12).

The unchanged collagen content in the colonic wall of parts II, III and IV indicates a balance between the synthesis and lysis of collagen. It can, however, not be deduced from these data if there is any change in collagen turnover.

In the rectum there are no marked changes in content and concentration of collagen or in tissue dry weight in the LR-group. This might be explained by the fact that the function of rectum is principally unchanged when the animals are fed LR-diet.

In the caecum there is a significant increase of collagen content and tissue dry weight in animals fed LR-diet. The collagen concentration, however, is decreased indicating a relatively larger increase in non-collagenous substances than in collagen. The increase of collagen content could be due either to an increase of collagen synthesis and/or a decrease of collagen lysis (10).

The findings in the present investigation indicate that in the colon relative bowel rest with low residue diet primarily affects the non-collagenous substances while collagen

content remains unchanged. In the caecum both the collagen and the non-collagenous substances increase. Thus the pattern of change in collagen and in non-collagenous substances varies in different parts of the large intestine. The difference may reflect the response to altered intestinal content. A more detailed analysis of collagen response to bowel rest requires studies of collagen synthesis or lysis.

ACKNOWLEDGEMENT

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II

THE EFFECT OF RELATIVE BOWEL REST ON COLLAGEN METABOLISM
AND SUTURE HOLDING CAPACITY IN THE COLONIC WALL

Studies in the rat

Peter Blomquist, Hasse Jiborn & Bengt Zederfeldt

ABSTRACT

In a previous experimental study it was shown that bowel rest with low residue diet led to reduction of the non-collagenous substances in the intact, unoperated colon, while the collagen content was unchanged. In the caecum both the collagen content and the non-collagenous substances were markedly increased.

In the present investigation the effect of relative bowel rest on collagen synthesis in the colonic wall was studied. Further, eventual influence on mechanical properties was evaluated. It was found that bowel rest led to a marked decrease of collagen and protein synthesis in the colon. In the caecum there was an unchanged collagen synthesis but an increased protein synthesis. It can be deduced that lysis of collagen is decreased in all parts of the colon. The depression of collagen turnover in the colonic wall was not accompanied by measurable changes in mechanical strength. It remains to study if the observed changes in collagen metabolism do affect colonic healing.

INTRODUCTION

Enteral nutrition with chemically defined diets has been introduced in clinical practice to give nutritional support while providing colonic preparation for surgery (5,6,8,10,14). These diets are low of residues and reduce the colonic content which permits the bowel to rest.

In a previous experimental study we found (3) that relative bowel rest with low residue diet in the intact, unoperated colon leads to decrease of non-collagenous substances while collagen content remains unchanged. However, the pattern of change in collagen and non-collagenous substances varied in different parts of the large intestine after institution of bowel rest. In the caecum both the collagen content and the non-collagenous components increase.

The present study was performed to investigate further the effect of relative bowel rest on collagen metabolism by determination of collagen synthesis in the colonic wall and to evaluate an eventual influence on mechanical properties.

MATERIAL AND METHODS

Thirty Wistar male rats weighing about 200 grams were used. They were randomly allocated to two groups that were kept in separate cages, receiving their respective diet and water ad libitum. One group (LR-group) was fed a fibre free, chemically defined diet (Biosorbin^R MCT, 100 g/100 g water) and the other group (SL-group) was given standard laboratory diet that contains 4 weight per cent fibres. After the study period of 11 days on the respective diet, the animals were sacrificed with an overdose of ether.

Experiment I (collagen synthesis)

Eight rats were used in each group. Twenty-four hours prior to sacrifice the animals were given 200 μ Ci L-proline (2-3-4-5-³H) (New England Nuclear, specific activity 100-120 Ci/mmol) intravenously in the tail. After sacrifice the colon and rectum were removed and divided in 5 defined parts (Fig. 1).

The incorporation of ³H-proline into proteins and collagen was determined according to Juva and Prockop (11) as applied in colonic healing by Jiborn et al. (9). The total radioactivity and the radioactivity of hydroxyproline (hypro) in each part were measured in a liquid scintillation counter (LKB Wallac 81000). Measurements of dry weight and hydroxyproline reported in a previous paper (3) were used for calculating the total radioactivity (total dpm³H/g dry weight) taken as a measure of protein synthesis and the specific activity of ³H-hydroxyproline (dpm³H-hypro/ μ mol hypro) which gives a measure of the newly synthesized collagen.

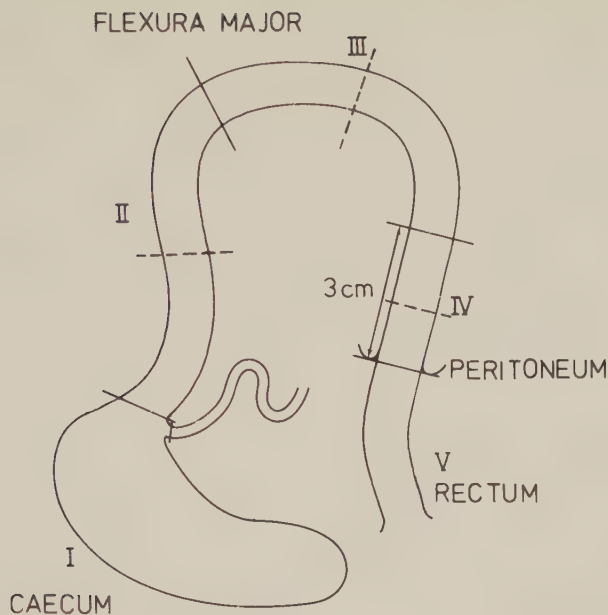


Fig. 1. Sites of colonic parts for analysis (I-V).

I: Caecum.

II: Pars proximalis coli to flexura major.

III: Pars distalis coli to 3 cm above the peritoneal reflection.

IV: Pars distalis coli below part III to the peritoneal reflection.

V: Rectum.

Dotted lines indicate places for determination of suture holding capacity (SHC).

(Nomenclature according to Lindström et al.(13)).

Experiment II (mechanical strength)

Seven rats were used in each group. After sacrifice the colon was removed and transected on three standardized places located in the middle of the colonic parts II, III and IV according to Figure 1. After transection a standardized end-to-end anastomosis was made with 4 interrupted sutures through the full thickness of the wall using 7-0 polypropylene as suture material. Segments about 3 cm in length, with the anastomoses in the middle, were tested in a specially constructed tensiometer. The force at rupture was recorded and called suture holding capacity (SHC).

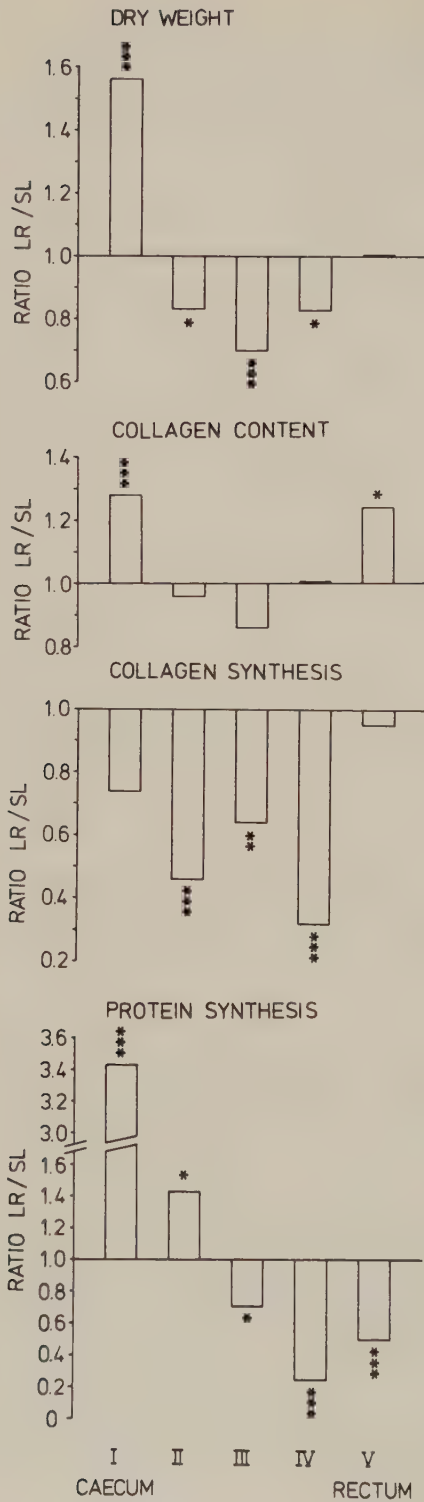


Fig. 2.

Tissue dry weight, collagen content (hydroxyproline content), collagen synthesis (specific activity of hydroxyproline) and protein synthesis (total radioactivity) in the different colonic parts 11 days on low residue diet (LR) expressed as a ratio to standard laboratory diet (SL). Asterisks denote level of statistical significance (see Statistical methods).

Statistical methods

In the statistical analysis of the results the mean (M), the standard deviation (SD) and the standard error of the means (SEM) were calculated. Comparisons of the means were carried out by Student's t-test for unpaired observations. Probability levels are represented by the following signs: *= $p < 0.05$; **= $p < 0.01$ and ***= $p < 0.001$.

RESULTS

Experiment I

The total radioactivity and the specific activity of hydroxyproline are given together with the values of dry weight and hydroxyproline content in the different parts of the colonic wall in Figure 2. All values are expressed as a ratio between the LR- and SL-groups. The total radioactivity is significantly increased in the caecum (part I) and pars proximalis coli (part II) but decreased in pars distalis coli (parts III and IV) and rectum (part V) in animals fed LR-diet. The specific activity of hydroxyproline is significantly reduced in the colon (parts II, III and IV) but unchanged in caecum (part I) and rectum (part V).

Experiment II

The values of suture holding capacity (SHC) of the colonic parts II, III and IV are illustrated in Figure 3. The differences between the means of SHC of the two groups are not significant.

DISCUSSION

In a previous experimental study we found (3) that bowel rest with a low residue diet (Biosorbin^R, MCT) (LR-diet) reduced the colonic and caecal contents by 90 and 45 per cent, respectively. This relative bowel rest led to decrease

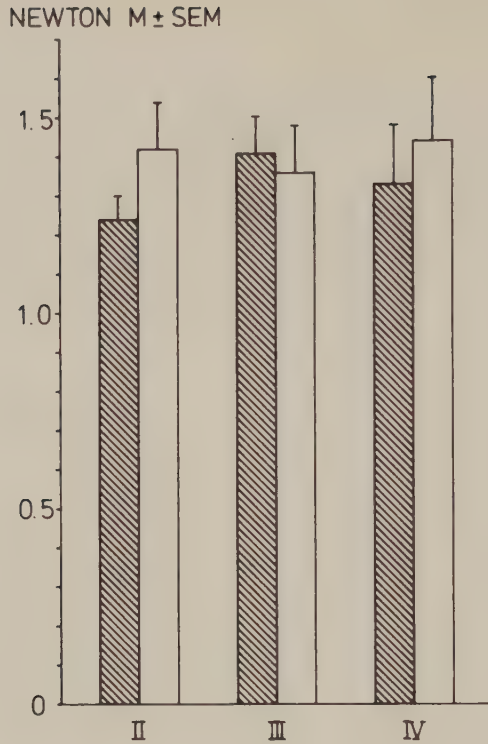


Fig. 3. Mechanical strength as suture holding capacity in the colonic parts II, III and IV after 11 days on low residue diet (shaded column) and standard laboratory diet (open columns).

of the dry weight of the colonic wall (parts II, III and IV) due to reduction of the non-collagenous substances, while the collagen content was unchanged. In the caecum, however, both the collagen content and the non-collagenous substances were markedly increased.

Detailed analysis of collagen metabolism requires determinations of collagen synthesis or lysis in addition to the net amount of collagen. In the present investigation the rate of collagen synthesis was studied using a pulse-labelling technique with ^3H -L-proline as precursor (9). The determination of ^3H -hydroxyproline is a measure of the rate of collagen synthesis. The total radioactivity reflects the syn-

thesis of all proteins including collagen. The specific activity of ^3H -hydroxyproline gives a measure of the newly synthesized collagen in relation to the total amount of collagen in each colonic part.

We have previously studied (18) the effect of diminished colonic content on anastomotic healing on the seventh post-operative day after four days preoperative treatment with LR-diet. To be able to make comparisons of values the eleventh day on LR-diet is used for investigation in the present study.

The collagen is not an inert substance but there is a continuous synthesis balancing the lysis of collagen always going on (12,15). In this study there is a marked decrease of collagen synthesis in the colon (parts II, III and IV). As collagen content in these parts remains unchanged (Fig. 2), lysis of collagen must be decreased. In the caecum (part I) and the rectum (part V), collagen synthesis is not significantly changed but collagen content is increased. Again this indicates decreased lysis of collagen. Thus, in animals on LR-diet all parts of the colon seem to have reduced collagen lysis.

A less likely explanation for the unchanged or increased collagen content is that the synthesis of collagen was increased during the early period on LR-diet. The differences between caecum and rectum on one side and the rest of the colon on the other is probably due to the fact that the caecum and the rectum still function as reservoirs for inspissated faeces in animals on LR-diet.

The protein synthesis is increased from caecum to flexura major (parts I and II) on LR-diet. In the rest of colon and rectum (parts III, IV and V) the protein synthesis is decreased. These findings offer an explanation for the changes found in the non-collagenous substances.

The depression of collagen turnover and the change in protein synthesis in the large intestine in a state of bowel rest with LR-diet represent adaptive changes in the bowel wall. The diminished colonic content may affect the collagen turnover in the bowel wall, for example by decreasing the luminal distension and/or by changing the colonic bacterial flora. Some authors have proposed that physical stimulation of food in the bowel lumen regulates cell replacement in the intestine (16). In some studies LR-diets reduced the colonic bacterial flora (4,17). However, in other studies on gastrointestinal disorders treated with LR-diet no change of the faecal flora has been found (1,2). The present study does not allow conclusions as regards those mechanisms.

The suture holding capacity, e.g. the strength of a newly made anastomosis, is almost completely dependent on the collagen in the submucous layer of the bowel wall (7). In the present paper the differences in collagen turnover between the LR- and SL-groups are not accompanied by significant changes in mechanical strength. A possible explanation for these findings is that collagen content and/or structural properties of collagen are of greater importance than collagen concentration and turnover for the mechanical strength measured as suture holding capacity.

The findings in the present investigation indicate that relative bowel rest with low residue diet is accompanied by a depression of collagen turnover in the colonic wall. These changes in collagen metabolism are not accompanied by measurable changes in mechanical properties. It remains to study if the observed changes in collagen metabolism do affect colonic healing.

ACKNOWLEDGEMENT

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III

THE EFFECT OF RELATIVE BOWEL REST ON HEALING
OF COLONIC ANASTOMOSES

Collagen synthesis and content in the colonic wall after
left colon resection and anastomosis in the rat

Peter Blomquist, Juhani Ahonen, Hasse Jiborn &
Bengt Zederfeldt

ABSTRACT

Collagen metabolism was studied in the colonic wall of rats after standardized resection and anastomosis. Diminished faecal loading was obtained by feeding rats low residue diet (Biosorbin^RMCT). In animals on low residue diet the post-operative increase of collagen synthesis and collagen content was on a lower level than in animals on standard laboratory diet. Furthermore, the increase was confined to the immediate anastomotic region and should represent changes in collagen caused by the operative trauma itself. It was concluded that the intraluminal content is an important factor in stimulating collagen turnover. The findings of lower collagen turnover in the anastomotic area in animals on low residue diet might be positive but could also imply impairment of healing. To evaluate this subject studies on mechanical strength of the anastomosis are necessary.

INTRODUCTION

Anastomotic leakage and dehiscence are complications of major clinical significance in gastro-intestinal surgery. In a multicentre study of 1466 patients who had undergone colonic resection there was a 13 per cent frequency of anastomotic leakage (Fielding et al., 1980) indicating that these problems are common. From clinical (Irvin & Goligher, 1973) as well as experimental studies (Jiborn et al., 1980b) it seems evident that faecal loading is a factor of major importance for the breakdown of left colonic anastomoses.

Faecal loading can be diminished by different means, one of which is bowel rest by low residue diet (Glotzer et al., 1973; Gurry & Ellis-Pegler, 1976; Hares & Alexander-Williams 1982).

Feeding rats low residue diet leads to a marked depression of collagen metabolism in the intact, unoperated colon

(Blomquist et al., 1984b). Low residue diet might thus mean impairment of healing after resection if formation of new collagen in the anastomosis is influenced in the same way.

The aim of the present investigation was to evaluate experimentally whether low residue diet and thereby diminished bowel content influences the collagen metabolism in the colonic wall after resection and anastomosis.

MATERIAL AND METHODS

Eighty-six Wistar male rats weighing about 200 g were used. Unoperated rats fed standard laboratory diet were used as controls. The other rats were randomly allocated to one of two groups:

LR-group, fed a fibre-free low residue diet (Biosorbin^R MCT, 100 g/100 g water) for 4 days prior to operation and throughout the experimental period, and

SL-group, fed standard laboratory diet, containing 4 weight per cent fibres.

In all animals in the LR- and SL-groups a left colon resection (Fig. 1) was performed under general anaesthesia (chloral hydrate: 25 mg/100 g body weight i.p.). The anastomosis was made using a single layer of continuous inverting suture of 7-0 silk.

Experiment I (Collagen content, days 2-14)

Altogether forty rats were studied in the LR- and SL-groups on days 2, 4, 7, and 14 postoperatively. The unoperated controls consisted of 22 rats. Blood drawn from the aorta was analyzed for haemoglobin (g/l) and albumin (g/l) concentration. After sacrifice the colon was removed and freed from adhesions. Standardized 10 mm segments (3_p and 3_d 5 mm) were taken on both sides of the anastomosis for analysis (Fig. 1).

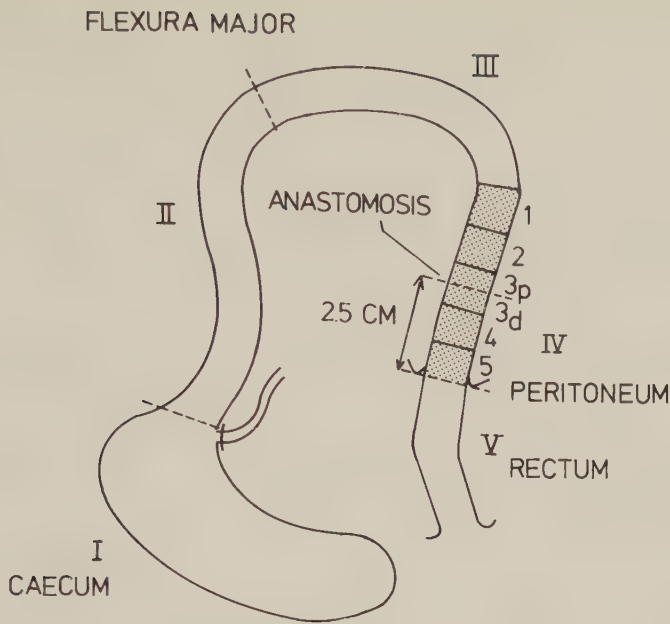


Fig. 1. Sites of anastomosis and studied colonic parts (I-V) and segments.

I: Caecum.

II: The proximal colon to the major flexure.

III: The distal colon to the anastomosis 2.5 cm above the peritoneal reflection.

IV: The distal colon below the anastomosis to the peritoneal reflection.

V: Rectum.

(Nomenclature according to Lindström et al., 1979).

Collagen was determined as hydroxyproline (hypro), according to the method described by Stegemann & Stalder (1967) and Pikkarainen (1968). The collagen content (μg hypro/segment) was calculated.

Experiment II (Collagen metabolism, day 7)

Eight rats were used in each group. The animals in the LR- and SL-groups were sacrificed seven days after resection and anastomosis. All rats were given $100 \mu\text{Ci}$ L-proline (2-3-4-5- ^3H) (New England Nuclear, specific activity 100-120 Ci/mmol) intravenously in the tail 24 hours prior to sacrifice.

After sacrifice anastomotic complications were registered. Colonic content was weighed. The colon and rectum were removed and divided in 5 defined parts (I-V). From these parts standardized, small segments were taken on both sides of the anastomosis (Fig. 1). Great care was taken to dissect free the anastomotic segments from adhesions.

Each colonic part and segment was dried to constant weight in an oven at 100° C and the dry weight was determined. Collagen was determined as hydroxyproline (hypro) as in experiment I. The collagen content (μg hypro/part and segment) and concentration (μg hypro/mg tissue dry weight) were calculated. The incorporation of ^3H -proline into collagen was determined according to Juva & Prockop (1966) as applied in colonic healing by Jiborn et al. (1980a). The radioactivity of hydroxyproline was measured in a liquid scintillation counter (LKB Wallac 81000). The specific activity of ^3H -hydroxyproline (dpm ^3H -hypro/ μmol hypro) which is a measure of the newly synthesized collagen was calculated.

Statistical methods

In the statistical analysis of the results the mean and the standard deviation were calculated. Comparisons of the means were carried out by Student's t-test for unpaired observations. Probability levels are represented by the following signs in figures:

* = $p < 0.05$; ** = $p < 0.01$ and *** = $p < 0.001$.

RESULTS

Experiment I

There were no significant differences in albumin or haemoglobin concentration between the LR- and SL-groups.

The collagen content in the anastomotic segments on post-

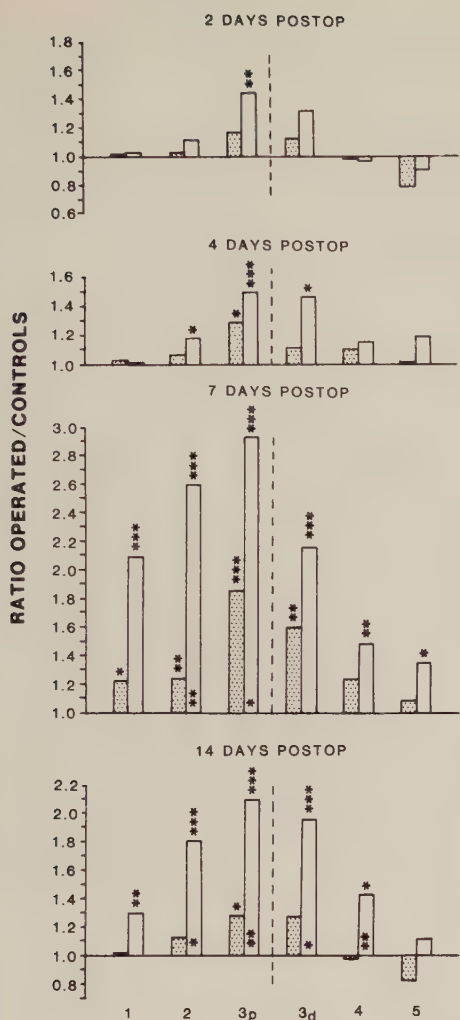


Fig. 2.

Collagen content in different colonic segments 2, 4, 7 and 14 days postoperatively in animals on low residue diet (shaded columns) and standard laboratory diet (open columns). The values are expressed as ratios to unoperated controls. Interrupted line indicates site of anastomosis. Asterisks denote level of statistical significance.

operative days 2, 4, 7 and 14 is given in Figure 2 as ratios to the unoperated controls. From the second to the seventh postoperative day there was a gradual increase of collagen content in the SL-group compared with the controls. Collagen content remained on a significantly higher level on day 14 but the difference against controls was less. The changes in the LR-group were principally the same but the increase in collagen content was on a lower level throughout. On the fourteenth day the collagen content in the LR-group was similar to the controls.

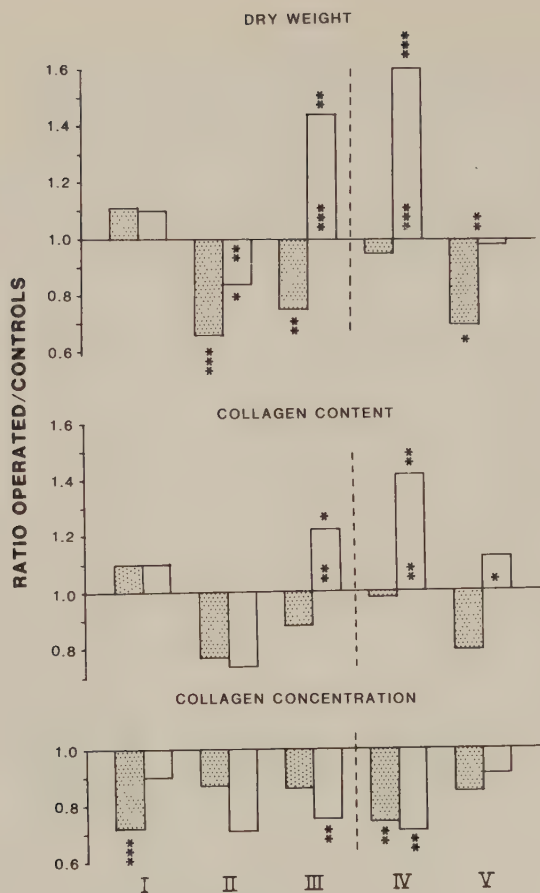


Fig. 3.

Dry weight, collagen content and collagen concentration in different colonic parts 7 days postoperatively in animals on low residue diet (shaded columns) and standard laboratory diet (open columns). The values are expressed as ratios to unoperated controls. Interrupted line indicates site of anastomosis. Asterisks denote level of statistical significance.

Experiment II

There were no gross anastomotic complications in either group. In the LR-group there were practically no adhesions to the anastomotic region but in the SL-group such adhesions were common. The weight of the colonic content was significantly lower ($p < 0.001$) in the LR-group on the seventh postoperative day than in the SL-group and the controls (0.7 ± 0.4 ; 2.1 ± 0.6 and 2.0 ± 0.4 g, respectively).

The dry weight and collagen of the colonic parts and anastomotic segments are given in figures as ratios to the unoperated controls.

RATIO OPERATED/CONTROLS

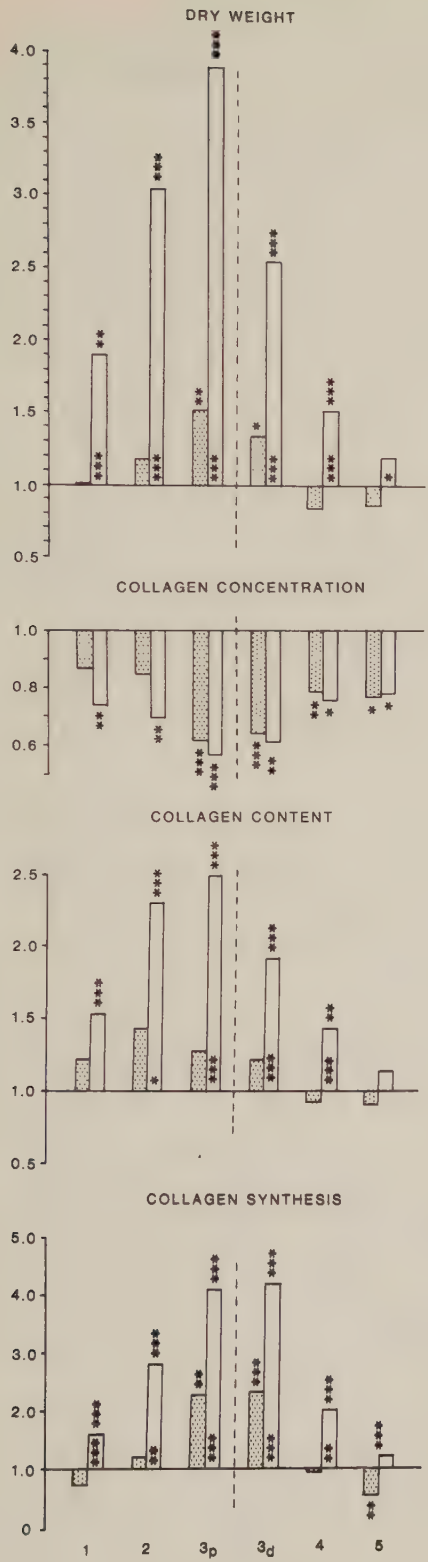


Fig. 4.

Dry weight, collagen concentration, collagen content and collagen synthesis in different colonic segments 7 days postoperatively in animals on low residue diet (shaded columns) and standard laboratory diet (open columns). The values are expressed as ratios to unoperated controls. Interrupted line indicates site of anastomosis. Asterisks denote level of statistical significance.

The dry weight (Fig. 3, 4) was significantly increased in the SL-group in parts III and IV compared with the unoperated controls. This increase was most pronounced in the close vicinity of the anastomosis, e.g. segments 2, 3_p and 3_d . In the LR-group the dry weight was significantly lower than in the SL-group in all the colonic parts and segments, except in the caecum. In fact the dry weight was decreased compared with the controls. Increase of dry weight was recorded only in the two anastomotic segments in the LR-group.

The collagen content (Fig. 3, 4) was significantly increased in the SL-group in parts III and IV compared with the unoperated controls. The increase was most marked on the proximal side of the anastomosis. In segments 2 and 3_p the collagen content was thus more than double that of the controls. In the LR-group the collagen content was significantly lower than in the SL-group in parts III-V and not significantly different from the controls.

The changes in collagen concentration (Fig. 3, 4) were similar in the SL- and LR-groups. In parts III and IV the collagen concentration was decreased by about 25 per cent compared with controls. The most marked decrease was observed in the anastomotic segments 3_p and 3_d .

The collagen synthesis (Fig. 4) was significantly increased in the three segments proximal and in two segments distal to the anastomotic line in the SL-group compared with the controls. In segments 3_p and 3_d the increase was more than 4 times. In the LR-group the collagen synthesis was significantly lower in all segments than in the SL-group. In the LR-group the collagen synthesis was increased only in the two anastomotic segments (3_p and 3_d) compared with the controls.

DISCUSSION

Changes in collagen metabolism after intestinal surgery is of interest since the mechanical strength of the bowel wall and its suture holding capacity is almost completely dependent on the collagen in the submucosal connective tissue layer (Halsted, 1887).

Detailed analysis of collagen metabolism requires determination of collagen content and collagen synthesis or lysis. In this study collagen synthesis in different segments of the colon was studied by a pulse-labelling technique using ^3H -L-proline as precursor (Jiborn et al., 1980a). Both the content and the concentration of collagen were determined in anatomically defined colonic parts as well as in smaller but well standardized colonic segments. The results from the parts and the segments correlated well, indicating that the data from the smaller colonic segments are representative.

The colonic content was markedly reduced when animals were fed low residue diet. Most of the remaining content was located in the caecum. The weight of the content in the distal colon was not specifically determined, but on inspection this part of colon was practically empty, which is consistent with previous reports on bowel rest (Blomquist et al., 1984a).

The present investigation confirms previous findings (Jiborn et al., 1980a) of marked and asymmetrical changes in collagen metabolism in the colonic wall after resection and anastomosis in animals on standard laboratory diet and shows that low residue diet changes this pattern. In operated animals on low residue diet the collagen content was regularly on a lower level than in animals on standard laboratory diet. The postoperative increase of collagen content in animals on low residue diet was confined to the immediate anastomotic region. Furthermore the increase of collagen content

was of shorter duration, returning to normal on the fourteenth postoperative day.

The reduction of collagen concentration in the anastomotic region is a result of smaller increase in collagen than in non-collagenous substances. Thus, the collagen concentration does not represent the actual collagen amount.

Clear differences in synthetic capacity of collagen were observed between the two groups of animals. Collagen synthesis increased less in animals on low residue diet than in animals on standard laboratory diet. While extensive changes in collagen synthesis were found after surgery in animals on standard laboratory diet, changes were limited to the immediate anastomotic segments in animals on low residue diet. The changes in collagen turnover and in non-collagenous components after colonic surgery in animals on low residue diet are consistent with the marked depression of collagen metabolism found in previous studies on the effect of bowel rest on the intact, unoperated colon (Blomquist et al., 1984b).

The factors that trigger changes in collagen turnover after colonic surgery are not fully known but it seems clear that collagenolytic enzymes are involved. Hawley et al. (1970) reported an increased collagenase activity in the entire gastrointestinal tract after colonic surgery. From the present data it is not possible to draw conclusions regarding lysis of collagen.

On the basis of the present findings it is reasonable to assume that the intraluminal content is an important factor in stimulating collagen turnover. The observed small changes in collagen metabolism in animals on low residue diet should then mainly represent changes in collagen caused by the operative trauma itself.

It should be noted that the data from the immediate anastomotic segments represent the deposition of collagen both in

the healing wound and in the adjacent bowel wall. The techniques used in this experiment do not allow measurement of collagen deposition solely in the anastomotic line. If, however, our findings of lower collagen turnover, in the anastomotic area in animals on low residue diet, represent changes in the anastomotic line, this might imply impairment of healing. It is, however, also possible that the altered collagen response should be seen as a positive factor. To evaluate this studies on mechanical strength of the anastomosis are necessary.

ACKNOWLEDGEMENT

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IV

THE EFFECT OF RELATIVE BOWEL REST ON HEALING OF COLONIC ANASTOMOSES

Breaking strength and collagen in the colonic wall following
left colon resection and anastomosis in the rat

Peter Blomquist, Hasse Jiborn & Bengt Zederfeldt

ABSTRACT

The breaking strength of anastomoses in the left colon in the early phase of healing was studied in rats fed low residue diet (Biosorbin^RMCT) and the results were compared to those from rats fed standard laboratory diet. Further, eventual correlation between mechanical strength development and collagen content in the colonic wall around the anastomosis was evaluated.

The anastomotic strength with sutures in place decreased by approximately 30 per cent of the immediate postoperative value during the first two days in both groups of animals. There was no correlation between changes in anastomotic strength and collagen content at that time. After the second day there was a gradual increase of anastomotic strength, reaching the strength at day 0 after 7 days. The regain of strength was mainly due to collagen deposition in the anastomosis. Despite more collagen deposition in animals on standard laboratory diet the anastomoses had comparable strength development in the two groups. It was concluded that low residue diet does not impair the suture holding capacity or the anastomotic strength. Instead there was some evidence for a more uncomplicated healing when the bowel content was diminished.

INTRODUCTION

Adequate preoperative bowel preparation is essential to reduce anastomotic complications in colorectal surgery (Muir, 1968; Irvin & Goligher, 1973; Hares & Alexander-Williams, 1982). Enteral nutrition with chemically defined diets low of residues has been introduced to give nutritional support while providing colonic preparation for surgery (Glotzer et al., 1973; Gurry & Ellis-Pegler, 1976).

We have previously reported (Blomquist et al., 1984a) that diminished colonic content, obtained by feeding rats low residue diet (Biosorbin^R MCT) results in a marked depression of collagen turnover in the colonic wall. This might mean impairment of healing of an anastomosis since collagen formation is one of the central factors for strength development but could as well be a positive factor (Blomquist et al., 1984b).

The purpose of the present study was to investigate the effect of relative bowel rest on the breaking strength of anastomoses in the left colon in the early phase of healing and to evaluate whether the mechanical strength of the anastomosis correlates to collagen content in the colonic wall.

MATERIAL AND METHODS

One hundred and twelve Wistar male rats weighing about 200 grams were used. They were randomly allocated to one of two groups. One group (LR-group) had free access to water and a low residue diet (Biosorbin^R MCT 100 g/100 g water) for four days preoperatively and during the experimental period. The other group (SL-group) had free access to standard laboratory diet and water.

In general anesthesia by intraperitoneal injection of chloralhydrate (25-30 mg/100 g body weight) a low median abdominal incision was performed. One centimeter of the left colon 2.5 cm above the peritoneal reflection was resected. A standardized anastomosis was made using a single layer of continuous suture with 7-0 silk. Groups of animals were sacrificed by an overdose of ether directly after the anastomosis was made (day 0) and on the first, second, third, fourth and seventh day postoperatively.

Gross appearance and mechanical strength determination

Perianastomotic abscesses were noted. The diameter of the colon proximal and distal to the anastomosis was determined with a caliper. The left colon was dissected free along the mesenteric border and adhesions were carefully removed from the anastomosis.

On day 0 to 4 the sutures were left in place. On the seventh postoperative day the suture in the SL-group had disappeared or hang loose in the colonic lumen. To have comparable conditions before strength testing the suture material was therefore removed in all animals on the seventh day. The anastomotic strength was tested by means of a specially constructed tensiometer which provided constantly increasing force. The force at rupture was recorded.

Collagen determinations

After the mechanical strength tests 5 mm segments on each side of the anastomosis were taken for analysis. Collagen was determined as hydroxyproline (hypro), according to the method described by Stegemann and Stalder (1967) and Pikkarainen (1968). The collagen content (μg hypro/part) was calculated.

Statistical methods

In the statistical analysis of the results the mean, the standard deviation and the standard error of the means were calculated. Comparisons of the means were carried out by Student's t-test for unpaired observations. Probability levels are represented by the following signs in figures: * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$.

RESULTS

The colonic diameter on both sides of the anastomoses on day 0 to 4 is illustrated in Figure 1. The bowel was wider on both sides of the anastomosis in the SL-group than in the

COLONIC
DIAMETER (MM)

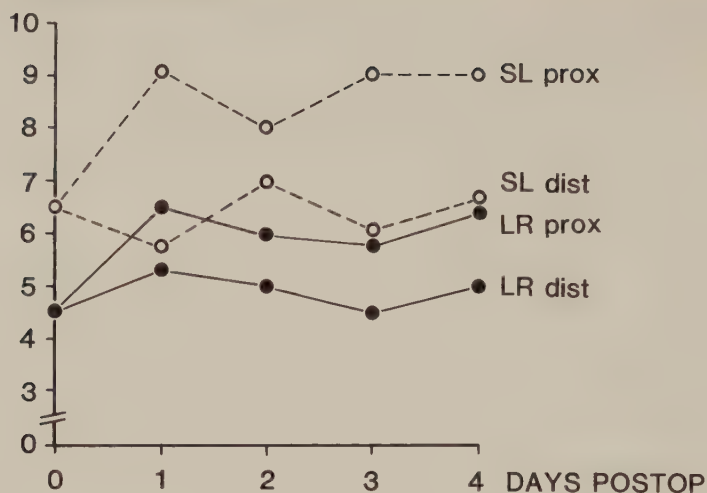


Fig. 1. Colonic diameter proximal and distal to the anastomosis on day 0 to 4 postoperatively in rats on low residue diet (● LR) and standard laboratory diet (○ SL).

TABLE I

BREAKING STRENGTH TESTS

Number of animals [excluded ()]:

	LR-group	SL-group	
DAY: 0	16	16	
1	8	8	
2	8	8	
3	7 (1)	6 (2)	
4	6 (2)	5 (3)	
7	8	7 (1)	
	53 (3)	50 (6)	TOTAL : 103 (9)

TABLE II

COLLAGEN DETERMINATIONS

Number of animals [excluded ()]:

	LR-group	SL-group	
DAY: 0	16	16	
1	8	8	
2	4 (4)	4 (4)	
3	7	6	
4	4 (2)	4 (1)	
7	8	7	
	47 (6)	45 (5)	TOTAL : 92 (11)

LR-group and in both groups wider on the proximal than on the distal side.

Nine animals had small perianastomotic abscesses. Eight of these were found on the third and fourth postoperative day (three in the LR-group and five in SL-group). All nine abscesses were considered as anastomotic complications and these animals were excluded from further study (Table I). Adhesions to the anastomotic region were more common in the SL-group. On the seventh day five out of seven animals in the SL-group showed marked adhesions but none of the eight animals in the LR-group had adhesions.

The breaking strength of the colonic anastomoses in the two groups are illustrated in Figure 2. In both groups there was a significant decrease of breaking strength on the first two postoperative days. From that time there was a significant ($p < 0.001$) gain in strength to the seventh postoperative day when breaking strength had reached the values of day 0.

In all tests the break occurred in the anastomotic line. The sutures came loose from the proximal side of the anastomosis in 27 out of 48 (56%) animals on day 0 and 1 and in 35 out of 40 (88%) on day 2 to 4.

For determination of collagen 92 animals were used. Nine animals with abscesses and 11 animals in which samples were lost because of technical failures were excluded. The number of animals in each subgroup is given in Table II.

There were no statistical differences in collagen content between the proximal and distal anastomotic segments. The sum of the two segments is therefore presented in the following text.

The collagen content in the anastomotic segment for the two groups is given in Figure 3 as ratios to the corresponding values on day 0. From the third day on there was an increase

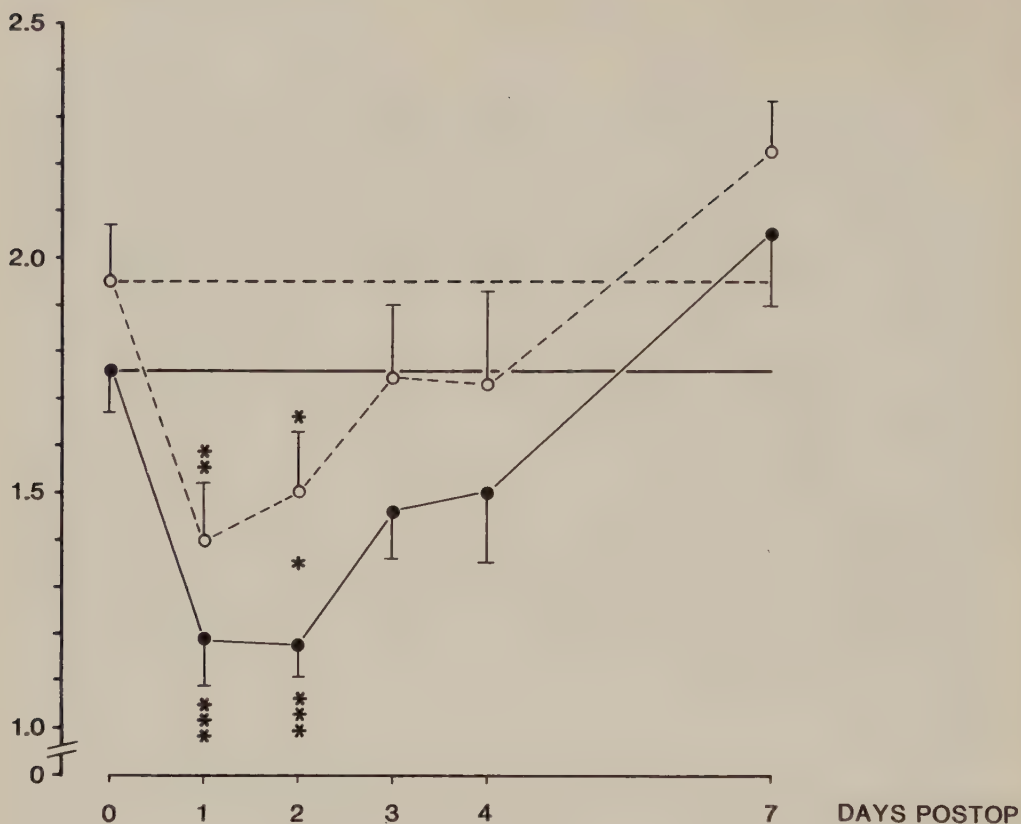


Fig. 2. Breaking strength of anastomoses in the left colon on the postoperative days 0 to 4 with sutures and on day 7 without sutures in rats fed low residue diet (●) and standard laboratory diet (○). Asterisks denote level of statistical significance.

of collagen content in the SL-group amounting to about 2.5 times on day 7. In the LR-group there was a significant decrease of collagen content on the second day but from the fourth day on an increase was found amounting to about 1.4 times on day 7, which was significantly lower than that observed in the SL-group.

COLLAGEN CONTENT

RATIO to values on day 0

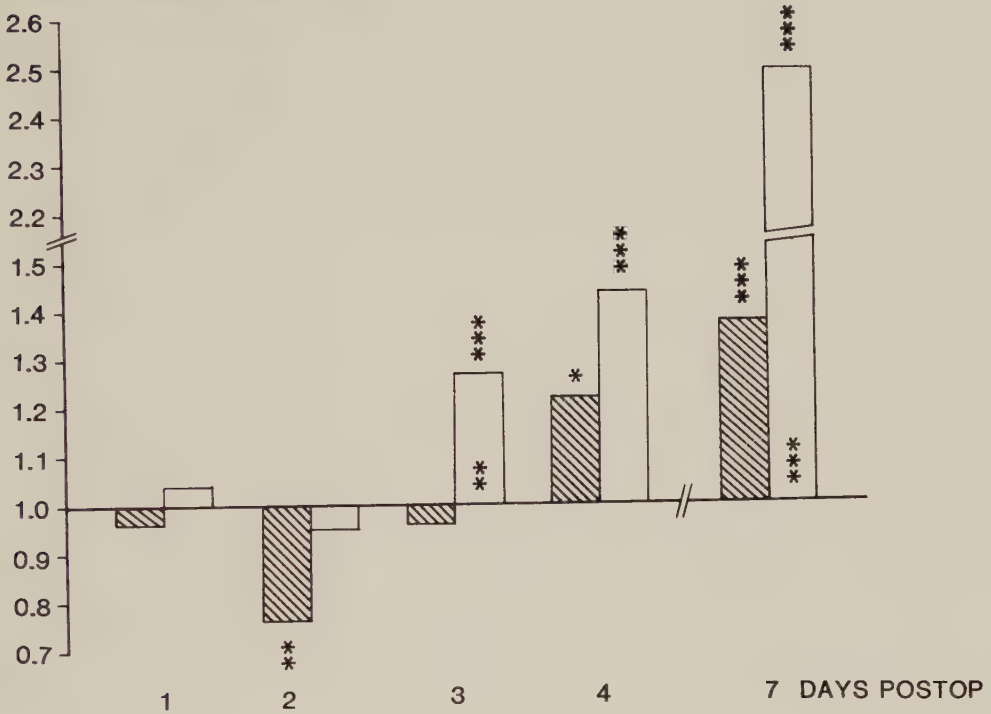


Fig. 3. Collagen content in the anastomotic segments of the colon on the postoperative days 1-4 and 7 in rats fed low residue diet (shaded columns) and standard laboratory diet (open columns). The values are expressed as ratios to the corresponding values on day 0. Asterisks denote level of statistical significance.

DISCUSSION

The development of strength in colonic anastomoses has been extensively studied (Fellows et al., 1951; Herrmann et al., 1964; Cronin et al., 1968; Irvin & Hunt, 1974; Wise et al., 1975; Jiborn et al., 1978a; Jiborn et al., 1978b). Conclusions regarding postoperative mechanical strength development are in most studies drawn from determination of breaking strength without sutures or from studies of bursting pressure. In this study the mechanical strength was tested with sutures in place from day 0 to 4.

During the early phase of anastomotic healing the mechanical strength of an anastomosis depends almost entirely on suture strength and on the ability of the bowel wall to hold the sutures (Howes et al., 1929; Cronin et al., 1968). As new tissue bridges the defect the role of the sutures becomes successively smaller. Thus, evaluation of the importance of suture support is of interest during the first critical days of healing, when anastomotic dehiscence is most common.

In this investigation we have studied the strength of an anastomosis in the left colon during the first postoperative week. During the first two days the anastomotic strength decreased by approximately 30% compared to the strength of the newly made anastomosis. During these days the suture holding capacity of the intestinal wall thus decreased. There was no correlation between changes in anastomotic strength and changes in the collagen content. Since the suture holding capacity of the bowel wall is mainly due to collagen (Halsted, 1887) not only the content but also the quality of collagen in the tissue around the sutures must be of importance.

After the second day there was a gradual increase of anastomotic strength, reaching the strength at day 0 after 7 days. Some of the regain of anastomotic strength may be due to changes of the suture holding capacity of the colonic wall but formation of new tissue with collagen deposition in the anastomosis is probably the major factor. As in these experiments sutures are removed before the seven day test all strength at that time is due to new tissue.

It was regularly found that the sutures came loose from the proximal side of the anastomosis on day 2-4. This could imply that the tearing forces are greater on the proximal side or that the structure of collagen is more changed on that side of the anastomosis.

The passage of faecal pellets exerts a strain on the anastomosis which probably explains why the sutures hang loose or had disappeared in animals on standard laboratory diet while they stayed intact in animals on low residue diet. Gross complications did not differ significantly between the two groups of animals. Anastomotic adhesions, however, were common in animals on standard laboratory diet but were absent in animals on low residue diet. This suggests that the diminished bowel content might give a more uncomplicated wound healing.

Jiborn et al. (1980) found that anastomotic complications were related to an enhanced turnover rate of collagen. We have previously reported (Blomquist et al., 1984b) that diminished bowel content decreased the collagen turnover in the anastomotic region. This further supports the assumption of a more uncomplicated wound healing after feeding animals low residue diet.

The anastomoses had comparable strength development in the two groups despite more collagen deposition in animals on standard laboratory diet than in those on low residue diet. This indicates either that the quality of collagen formed is different in the two groups or that the increased collagen amount is located mainly outside the anastomotic line.

No studies of the strength of intact intestinal wall were performed in this investigation. If the data from Jiborn et al. (1978b) for colonic strength (6.1 N for the left colon) are used for comparison it can be calculated that the strength of a newly made anastomosis is only 30% of that of the intact bowel wall and that the strength of an anastomosis at its lowest point on day 2 postoperatively is approximately 20% of the strength of the intact intestinal wall. The lowest values of strength of an anastomosis at the second day were on the same level as those found for small intestine by Jönsson et al. (1983). They, however, found a different time schedule for changes in anastomotic strength.

A close comparison would require identical suture technique which was not the case in these two studies.

The findings in the present study indicate only minor differences in anastomotic strength during the early phase of colonic healing in animals on low residue diet and on standard laboratory diet. Thus the reduced collagen turnover rate, as a consequence of low residue diet, does not impair the suture holding capacity or the anastomotic strength in the early healing phase. Instead there is some evidence for a more uncomplicated anastomotic healing when the bowel content is diminished.

ACKNOWLEDGEMENT

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V

THE EFFECT OF DIVERTING COLOSTOMY ON COLLAGEN
METABOLISM IN THE COLONIC WALL

Studies in the rat

Peter Blomquist, Hasse Jiborn & Bengt Zederfeldt

INTRODUCTION

Diversion of the faecal content by a colostomy is widely used to decompress the left colon in obstruction or to prevent complications in colorectal anastomoses. However, some authors have in clinical studies reported high incidence of anastomotic leakage despite proximal colostomy (1,2).

The aim of the present study was to evaluate experimentally whether faecal diversion influences collagen metabolism in the colonic wall. This might be of interest since the mechanical strength of the intact bowel wall and its suture bearing capacity is mainly dependent on the collagen in the submucosal layer (3).

MATERIAL AND METHODS

Forty-eight Wistar Male rats weighing about 200 g were used. The animals had free access to standard laboratory pellets and water. They were randomly allocated to one of two operations: colostomy or shamoperation.

In both groups of animals a median laparotomy was carried out under general anaesthesia (chloral hydrate; 25 mg/100 g body weight i.p.). In the colostomy group the colon was transected at the major flexure (Fig. 1). The proximal end was brought out as a terminal colostomy in the upper part of the incision. The bowel wall was fixed to the fascia and skin with 7-0 polypropylene sutures. The distal end was closed with the same suture material. The shamoperated animals were submitted to laparotomy and mobilisation of the flexure.

The postoperative weight development was recorded for all animals. Eight animals from each group were sacrificed on postoperative days 4, 11 and 28. Blood drawn from the aorta under ether anaesthesia prior to sacrifice was analyzed for haemoglobin (g/l) and albumin (g/l).

After sacrifice the colon and rectum were removed and divided in four parts, called I, II, III+IV and V, for analysis (Fig. 1). The designation III+IV was used in agreement with previous studies on related subjects.

Animals sacrificed on the 11th day were used for determination of collagen and protein synthesis. They were given 200 μCi ^3H -proline (2-3-4-5- ^3H -L-proline, New England Nuclear, specific activity 100-120 Ci/mmol) intravenously in the tail 24 hours prior to sacrifice.

The dry weight (mg) of each colonic part was recorded. Collagen was determined as hydroxyproline (hypro), according to the method described by Stegemann and Stalder (4) and Pikkarainen (5). The collagen content (μg hypro/part) and con-

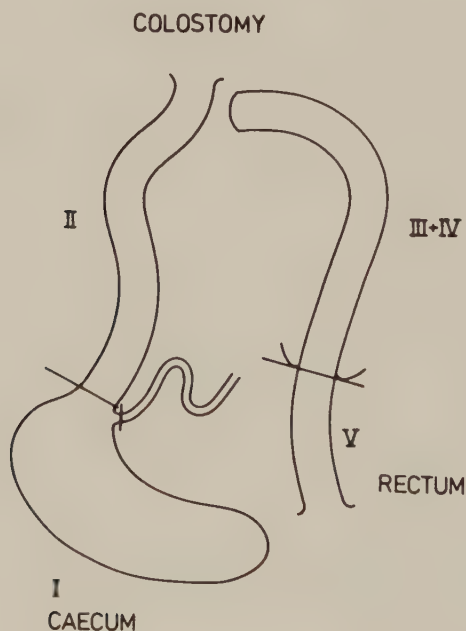


Fig. 1. Sites of colostomy and studied colonic parts.

I: Caecum.

II: The proximal colon to the major flexure.

III+IV: The distal colon to the peritoneal reflection.

V: Rectum.

(Nomenclature according to Lindström et al. (15)).

centration (μg hypro/mg tissue dry weight) were calculated. The incorporation of ^3H -proline into proteins and collagen were determined according to Juva and Prockop (6) as applied in colonic healing by Jiborn et al. (7). The radioactivity was measured in a liquid scintillation counter (LKB Wallac 81000). The protein synthesis (total dpm ^3H /g dry weight) and the collagen synthesis (dpm ^3H -hypro/ μmol hypro) were calculated.

Statistical methods

In the statistical analysis of the results the mean (M), the standard deviation (SD) and the standard error of the means (SEM) were calculated. Comparisons of the means were carried out by Student's t-test for unpaired observations. Probability levels are represented by the following signs in figures: * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$.

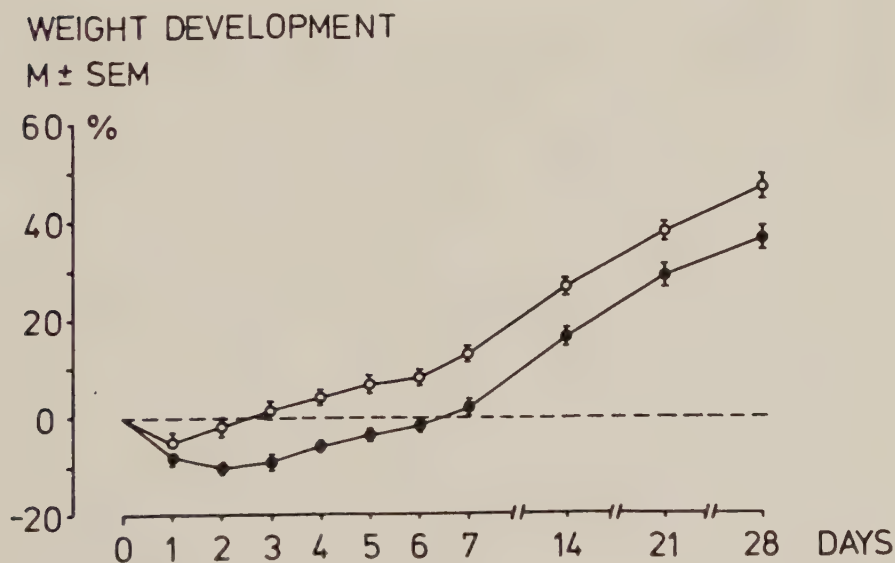


Fig. 2. Postoperative weight development in per cent of body weight for animals in the colostomy group (●) and in the sham-operated group (○).

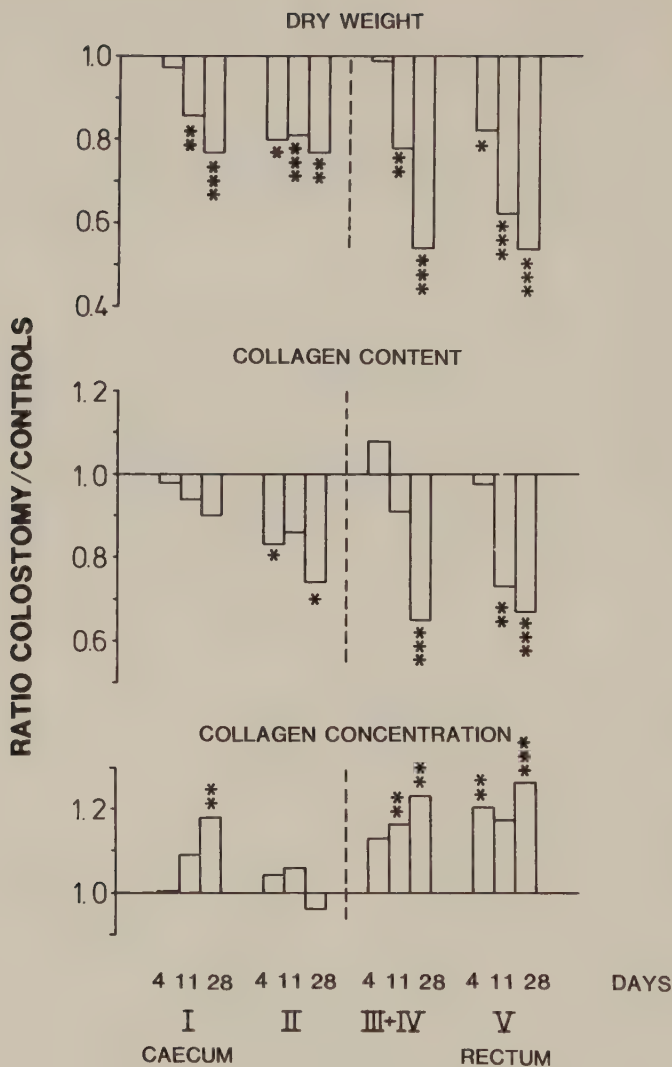


Fig. 3. Tissue dry weight, collagen content and collagen concentration in the different colonic parts 4, 11 and 28 days after operation. The values are expressed as ratios between the colostomy group and the sham-operated controls. Interrupted line indicates colostomy site. Asterisks denote level of statistical significance.

RESULTS

The postoperative weight development was delayed in the colostomy group (Fig. 2).

There were no significant differences of haemoglobin and albumin concentration in the two groups.

The dry weight of the colonic parts (Fig. 3,4) was significantly decreased both proximal and distal to the colostomy site compared with the controls. The decrease was most pronounced in the excluded parts after 28 days.

The collagen content (Fig. 3,4) was considerably decreased in the rectum 11 days after the operation and in both parts distal to the colostomy site after 28 days compared with the controls. Also in the functional part II there was a lower collagen content compared with the controls.

The collagen concentration (Fig. 3,4) was markedly increased in the two excluded parts compared with the controls. Proximal to the colostomy site the collagen concentration was virtually unchanged.

The collagen and protein synthesis on day 11 (Fig. 4) were significantly decreased distal but unchanged proximal to the colostomy site compared with the controls.

COMMENTS

The present study shows that faecal diversion leads to marked depression of collagen synthesis in the excluded colon. This was accompanied by a decrease of collagen content. The observed increase of collagen concentration despite decreased collagen content is explained by the considerable decrease of non-collagenous substances, which constitute the main part of the tissue dry weight. Thus, the collagen con-

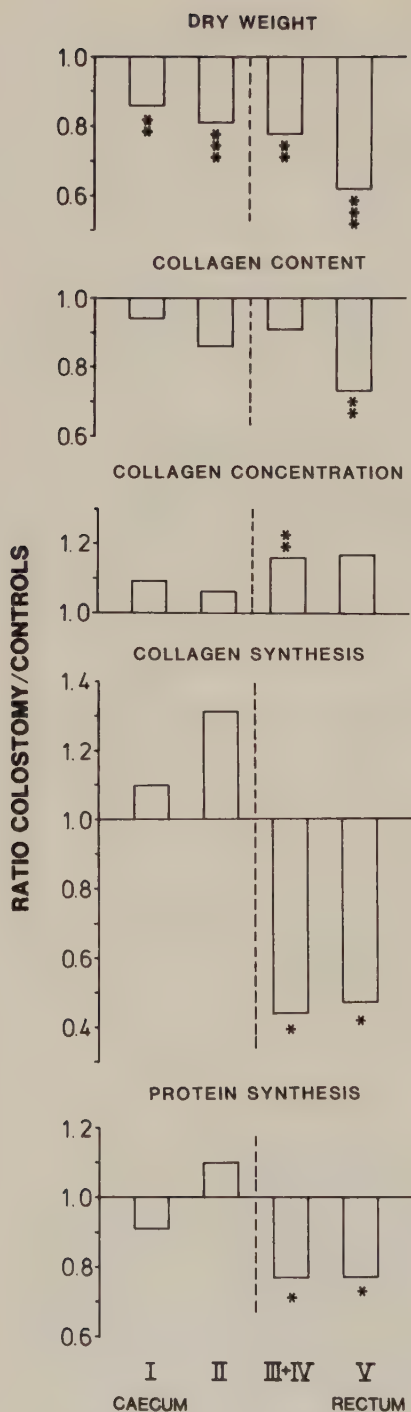


Fig. 4.

Tissue dry weight, collagen content, collagen concentration, collagen synthesis and protein synthesis in the different colonic parts on the 11th postoperative day. The values are expressed as ratios between the colostomy group and the sham-operated controls. Interrupted line indicates colostomy site. Asterisks denote level of statistical significance.

centration does not represent the actual collagen amount. The observed decrease of protein synthesis distal to the colostomy site offers an explanation to the changes found in the non-collagenous components.

The present findings are principally in agreement with the results from previous studies on the effect of low residue diet on the intact, unoperated colon (8). Relative bowel rest with low residue diet was also accompanied by a depression of collagen turnover in the colonic wall, but collagen content was unchanged. Total bowel rest, obtained by a colostomy seems to result in a more marked depression of collagen turnover, as the collagen content decreased in course of time.

Several factors are proposed to influence cell replacement in the colon, e.g. gastrin (9), bacterial flora (10) and physical stimulation of food residues in the bowel lumen (11). After total bowel rest by a diverting colostomy the left colon undergoes progressive atrophy, with loss of cells principally in the mucosal layer of the bowel (12-14). The factors that regulate the collagen turnover in the bowel wall are not known but the findings in the present study indicate that the stimulation of intraluminal bulk might be of importance.

The decrease of tissue dry weight and collagen content in the colonic part proximal to the colostomy is difficult to explain. These changes were less marked than in the excluded colonic parts. One explanation might be that the intraluminal pressure was affected proximal to the colostomy site. Other explanations, such as changes in bacterial flora, could be considered.

The observed differences in weight development were hardly of a magnitude to affect the parameters studied.

The findings in the present study indicate that faecal

diversion by colostomy is accompanied by a depression of collagen turnover in the wall of the excluded colon. It remains to be studied if these changes affect anastomotic healing and mechanical strength development following colonic surgery.

SUMMARY

In the present investigation the effect of total bowel rest by colostomy on collagen metabolism was studied. Faecal diversion led to a marked decrease of collagen and protein synthesis in the excluded colon. These changes were accompanied by a decrease in the amount of collagen and non-collagenous components. In the colonic part proximal to the colostomy less marked decrease of collagen content was observed. It was concluded that stimulation of intraluminal bulk might be of importance as a regulating factor for collagen turnover. It remains to study if the observed changes in collagen metabolism affect colonic healing.

ACKNOWLEDGEMENTS

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VI

THE EFFECT OF DIVERTING COLOSTOMY ON BREAKING STRENGTH
OF ANASTOMOSES AFTER LEFT COLON RESECTION

Studies in the rat

Peter Blomquist, Hasse Jiborn & Bengt Zederfeldt

INTRODUCTION

Resection and anastomosis of the left colon is associated with a high incidence of anastomotic failure (1). Diversion of the faecal content by a proximal colostomy is often used to prevent complications. However, considerable incidence of anastomotic leakage despite proximal diversion has been reported by several authors (1-3).

In a previous experimental study we reported (4) that faecal diversion by colostomy results in a marked depression of collagen metabolism in the wall of the excluded colon. Since collagen formation is of importance for intestinal wall strength and anastomotic strength development (5) faecal diversion might mean decreased suture holding capacity and delay of healing of an anastomosis.

The aim of the present study was to investigate the effect of a proximal diverting colostomy on suture holding capacity and on breaking strength of anastomoses in the excluded left colon.

MATERIAL AND METHODS

Forty-eight Wistar male rats weighing about 200 grams were used. The animals had free access to standard laboratory pellets and water. They were randomly allocated to one of four groups treated as shown in Table I.

TABLE I

	Operation	Test day 7
Group A (8)	Sham	Suture holding capacity
Group B (8)	Colostomy	---
Group C (16)	Colostomy & resection + anast.	Anastomotic strength
Group D (16)	Resection + anast.	---

(In parenthesis number of animals)

In all groups of animals a median laparotomy was carried out under general anesthesia (chloral hydrate; 25 mg/100 g body weight i.p.). In group A the colon was mobilized. In groups B and C the colon was transected at the major flexure (Fig. 1). The proximal end was brought out as a terminal colostomy in the upper part of the incision. The bowel wall was fixed to the fascia and skin with 7-0 polypropylene sutures. The distal end was closed with the same suture material after emptying the faecal content in the excluded colon. In groups C and D one cm of the distal colon 2.5 cm above the peritoneal reflection was resected. A standardized anastomosis was made using a single layer of interrupted sutures with 7-0 polypropylene.

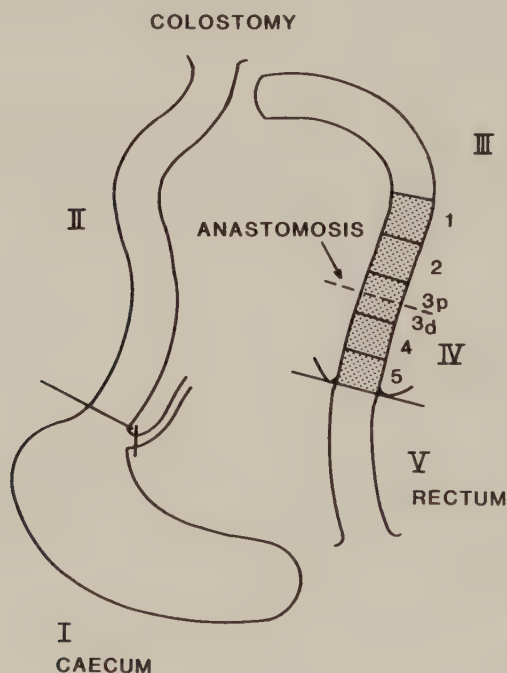


Fig. 1. Sites of colostomy and studied colonic parts (I-V) and segments (1-5).

I: Caecum.

II: The proximal colon to the major flexure.

III: The distal colon to the anastomosis 2.5 cm above the peritoneal reflection.

IV: The distal colon below the anastomosis to the peritoneal reflection.

V: Rectum.

(Nomenclature according to Lindström et al. (13)).

NEWTON $\pm$ SEM

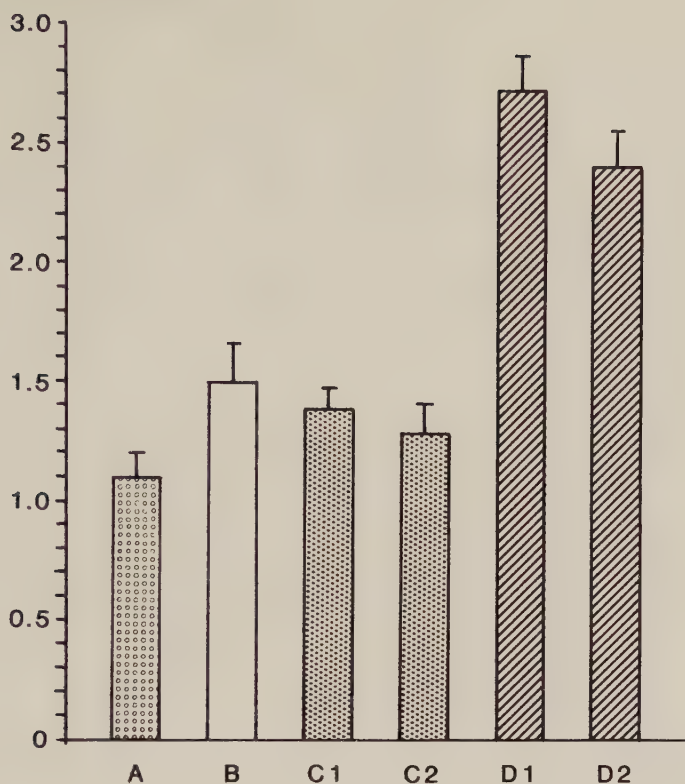


Fig. 2. Suture holding capacity (A and B) and anastomotic strength on the seventh postoperative day with sutures (C₁, D₁) and without sutures (C₂, D₂). A = Shamoperated group; B = Colostomy group; C = Colostomy and resection group; D = Resection group.

The postoperative weight development was recorded for all animals. On the seventh postoperative day the animals were sacrificed by an overdose of ether. In groups C and D signs of anastomotic complications were registered.

Suture holding capacity was tested in groups A and B. A colonic resection and anastomosis was made just before sacrifice as described for groups C and D and its strength immediately determined.

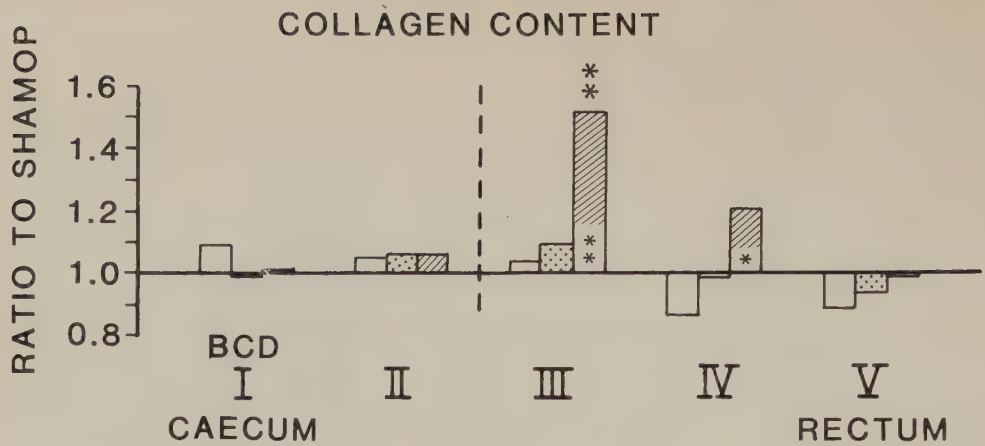


Fig. 3. Collagen content in different colonic parts on the seventh postoperative day in animals with colostomy (B), with colostomy and left colon anastomosis (C) and with left colon anastomosis (D). The values are expressed as ratios to sham-operated animals. Interrupted line indicates site of colostomy in group B and C. Asterisks denote level of statistical significance (those within columns indicate differences to group C).

Anastomotic strength was determined after 7 days of healing in groups C and D. In each group half of the animals were tested with sutures in place and the other half after removal of sutures in order to allow evaluation of the importance of suture support in a seven day old anastomosis.

Breaking strength of the colonic segment containing the anastomosis was made by a specially constructed tensiometer providing constantly increasing force. The force at rupture was recorded.

Collagen determination

After the mechanical strength tests the colon and rectum were divided in 5 defined parts (I-V). From parts III and IV standardized, 10 mm segments were taken on both sides of the anastomosis (anastomotic segments 3_p and 3_d were each 5 mm in length; Fig. 1).

Collagen was determined as hydroxyproline (hypro) in each colonic part and segment according to the method described by Stegemann & Stalder (6) and Pikkarainen (7). The collagen content (μg hypro/part and segment) was calculated.

Statistical methods

In the statistical analysis of the results the mean (M), the standard deviation (SD) and the standard error of the means (SEM) were calculated. Comparisons of the means were carried out by Student's t-test for unpaired observations. Probability levels are represented by the following signs: $*=p<0.05$; $**=p<0.01$ and $***=p<0.001$.

RESULTS

The postoperative weight gain was similarly delayed in groups B, C and D compared to the shamoperated group (A).

In group C no signs of anastomotic leakage were seen. In group D three out of sixteen animals showed signs of anastomotic leakage with small perianastomotic abscesses. These three animals were excluded from further study. Adhesions to the anastomotic region were not seen in group C but were common in group D.

The mean value of suture holding capacity (groups A and B) was approximately 35 per cent higher ($p<0.05$) in group B with colostomy than in group A (Fig 2).

The anastomotic strength was approximately 45 per cent lower ($p<0.001$) in group C than in group D (Fig. 2). The strength without sutures (C_2 and D_2) was not statistically different from that with sutures left in place (C_1 and D_1).

The collagen content in colonic parts and segments of the different groups of animals is given in fig. 3 and fig. 4 as a ratio to the shamoperated animals (A). In group B and C

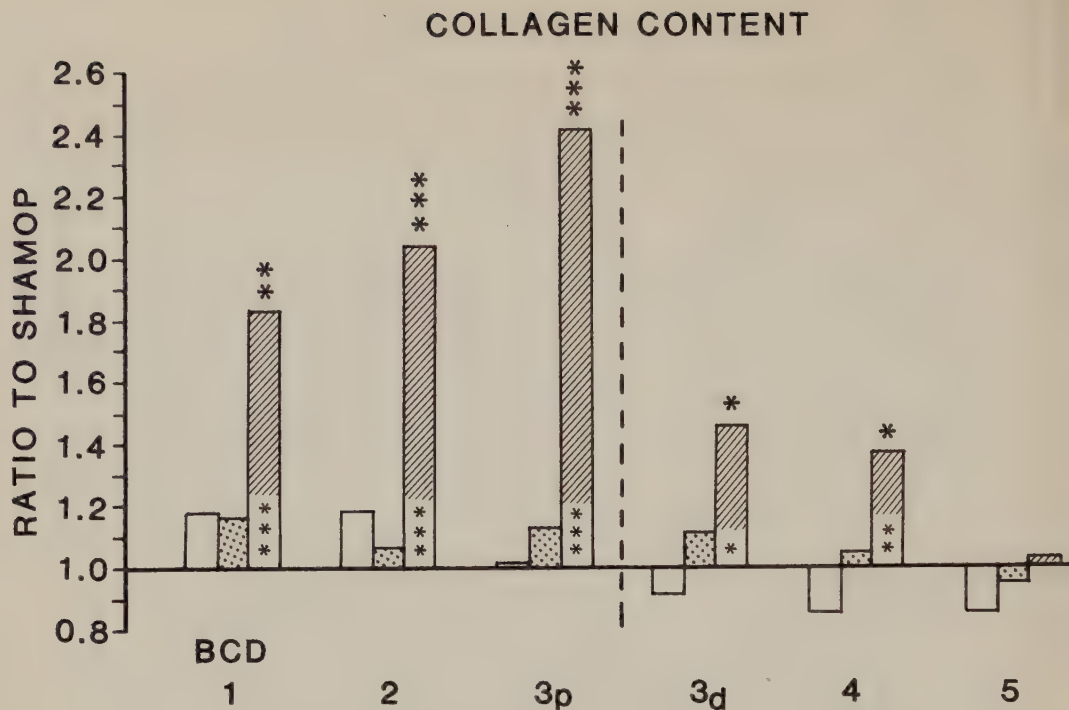


Fig. 4. Collagen content in different colonic segments on the seventh postoperative day in animals with colostomy (B), with colostomy and left colon anastomosis (C) and with left colon anastomosis (D). The values are expressed as ratios to shamoperated animals. Interrupted line indicates site of anastomosis in group C and D. Asterisks denote level of statistical significance (those within columns indicate differences to group C).

the collagen content was the same in all colonic parts and segments as in group A. In group D the collagen content was significantly higher in part III and in segments 1-4 than in group C.

DISCUSSION

In this investigation we have studied the suture holding capacity seven days after diverting colostomy as well as the strength of an anastomosis in the left colon with and without a proximal diverting colostomy. At this time formation

of new collagen in the anastomosis has begun (8-10). The anastomotic strength was tested both with and without sutures to evaluate the importance of suture support one week postoperatively.

The suture holding capacity, was approximately 35 per cent higher ($p < 0.05$) in animals with a one week old proximal colostomy than in shamoperated animals. Suture holding capacity is determined mainly by collagen (5). As the amount of collagen was found to be similar in the two groups it can be assumed that collagen quality is changed. The previously reported (4) reduced turnover rate of collagen in the excluded colon might be the basis for such a quality change.

The anastomotic strength on the seventh postoperative day was approximately 45 per cent lower ($p < 0.001$) in animals subjected to left colon resection with a proximal colostomy than in those resected but without a colostomy. This indicates that the presence of intraluminal content stimulates strength development of an anastomosis. Using the data from Jiborn et al. (11) for strength of the intact colonic wall (6.1 N for the left colon) it can be calculated that the strength of a one week old anastomosis in animals with and without a colostomy is only approximately 20 and 40 per cent, respectively, of that of the intact bowel wall.

The breaking strength was similar in each resected group with and without sutures in place. This suggests that suture support does not contribute to strength of colonic anastomoses one week after surgery, irrespective of a diverting colostomy or not.

The present investigation confirms previous studies (10) of marked increase of collagen content in the anastomotic region following colonic surgery. Faecal diversion, however, eliminates the postoperative collagen response. The findings are consistent with previous results of marked reduction of collagen turnover rate in the excluded colonic wall as a

consequence of proximal faecal diversion (4).

We have previously reported (12) that relative bowel rest, obtained by feeding rats low residue diet (Biosorbin^R MCT), led to an unchanged postoperative strength development, despite a marked depression of collagen turnover in the colonic wall. Total bowel rest, obtained by a colostomy, seems to result in a more marked decrease of collagen turnover and a delay of anastomotic strength development.

Gross anastomotic complications occurred in 3 animals without a diverting colostomy against none in animals with a colostomy. Furthermore, anastomotic adhesions were common in animals without a colostomy but were not seen in those with a diverting colostomy. This suggests that total bowel rest gives a more uncomplicated wound healing. It should be noted that the excluded bowel was emptied of faecal contents during operation, which might reduce the risk of anastomotic leakage.

The findings in the present study indicate that faecal diversion does not diminish the ability of the sutured bowel wall to withstand tearing forces. Instead there was an increase of suture holding capacity in the excluded colon seven days after diverting colostomy. However, total bowel rest led to a marked delay of anastomotic strength development. These differences in strength development were accompanied by a diminished collagen response in the anastomotic area after faecal diversion. If this represents an impaired healing or is a sign of less complicated healing remains to answer. The gross appearance of the anastomoses indicate that colostomy prevents complications like abscesses and adhesions.

SUMMARY

In the present investigation the effect of a proximal diverting colostomy on suture holding capacity and on anastomotic strength of the excluded left colon was studied. The suture holding capacity was increased seven days after faecal diversion. The anastomotic strength development, however, was significantly delayed. These differences were accompanied by a diminished collagen response in the anastomotic region after faecal diversion. This might suggest an impairment of healing in the excluded colon. The gross appearance of the anastomoses would, however, rather indicate that increased collagen formation and higher strength development in animals without colostomy is a result of more complicated healing.

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